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BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

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CONTENTS

	PAGE	RUSS. PAGE
New Targets for Medical Science	105	
PHYSIOLOGY		
Changes in the Bioelectrical Activity in Various Areas of the Cerebral Cortex in Dogs, During the Formation of a Temporary Connection. V. N. Dumenko	106	4
General and Local Changes in the Electroencecphalogram During the Formation of Conditioned Motor Reflexes in Man. Ts'u Chih-p'ing	110	8
The Functional Symmetry of the Cortical Part of the Auditory Analyzer. V. M. Mosidze	115	14
Representation of the Internal Organs in the Cerebral Cortex and Cerebellium of Cats and Dogs Communication IV. Cortical Representation of the Pelvic and Pudendal Nerves in Dogs. K. M. Kulanda	117	16
Changes in the Lability of Smooth Muscle During Stimulation of the Sympathetic and Parasympathetic Nerves. R. S. Orlov and Kh. S. Khamitov	122	22
The Demarcational Potential Difference as a Characteristic of the Changing State of Polarization of Skeletal Muscles at Different Age Periods. I. I. Gokhblit and I. A. Kornienko	125	26
The Action of Pituitrin on the Work of the Gastric Glands. A. S. Yampol'skii	129	32
The Relationship Between the Concentrations of Thyrotropic Hormone in the Hypophysis and the Peripheral Blood. R. D. Vyazovskaya and V. V. Mamina	133	36
PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY		
The Effect of Denervation of the Spleen on the Serum Vitamin B_{12} Level in Dogs. V. F. Markelova	137	41
Changes in Hemopoiesis in Congestive Splenomegaly With or Without Denervation of the Spleen. I. Ya. Ashkinazi	141	46
Characteristics of the Anemia Following Division of the Vagus Nerves. R. A. Durinyan	144	50
Hemopoietic Properties of the Sera of Healthy and Anemic Animals (Cats). E. L. Kan	148	55
Changes in the Peripheral Blood and Bone Marrow of Rabbits in Experimental Epilepsy. V. M. Karlinskii	153	61
The Nature of Spinal Shock Communication IV. Atony of the Motor Neuron in Spinal Shock. G. N. Sorokhtin, O. P. Minut-Sorokhtina, and Yu. B. Temper	156	65
The Parabiotic Nature of the Reaction of the Nervous System During the Action of Gas Gangrene Toxin. S. V. Magaeva	158	68
BIOCHEMISTRY AND BIOPHYSICS		
The Correlation Between the Active Concentration of Labeled Amino Acids by the Cells, and Their Inclusion in the Proteins of Tumors and Normal Tissues of Experimental Animals in Vitro. K. A. Perevoshchikova	162	73

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	PAGE	RUSS. PAGE
The Effect of Isohemotransfusion on the Metabolism of Adrenergic Substances in the Brain and Effector Organs. R. M. Glants	166	77
MICROBIOLOGY AND IMMUNITY		
The Effect of Pain Stimulation on the Phagocytic Power of the Leukocytes in Horses. A. F. Rusinov	169	81
The Effect of the Secretion of the Cutaneous Glands of the Toad on Certain Immunological Properties of Animals. V. I. Zakharov, N. V. Kolpikov and B. M. Kurtser	173	85
The Electrophysiological Analysis of the Action of Antigens on Angioceptors. A. N. Gordienko, V. I. Kiseleva, R. B. Tsynkalovskii, B. A. Saakov, Ya. I. Azhipa, A. V. Let'en, A. I. Egorov, L. N. Ochelenko, I. M. Bondarev, and L. I. Zhigalina	177	90
Changes in the Morphology of Connective Tissue Explantates in Rats Treated with Methylcholanthrene and Extract from a Human Lung Tumor. M. T. Mogila and Ya. V. Dobrynin	181	95
Tumors Induced by Methylcholanthrene in the Steppe Lemming. A Transmissible Strain of Lemming Sarcoma (LS). N. I. Bolonina	184	99
The Immunogenic Properties of Explantates of Brown-Pearce Rabbit Tumors When Cultivated in Human Serum. M. S. Lomakin	187	102
The Production of Specific Sera to Ehrlich's Mouse Carcinoma on the Basis of the Phenomena of Acquired Immunological Tolerance. Yu. N. Mol'kov	191	108
EXPERIMENTAL BIOLOGY		
Morphological Changes in Regenerating Rat Kidney at Long Intervals after Injury. G. G. Samsonidze	195	113
Morphological Reaction of Uterine Wall Tissues of the White Rat to a Foreign Body. Liu Tsien-Ch'ang	198	116
A New Head-Holder for Cats. R. A. Durinyan and A. I. Bartyzel'	201	121
A Simple Method for Phonocardiography in Small Laboratory Animals. I. F. Mineev and V. K. Sel'tser	203	123

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NEW TARGETS FOR MEDICAL SCIENCE

The recently published decree of the Central Committee of the CPSU and the Council of Ministers of the USSR, "Measures for the further improvement of the medical service and the care of the health of the population of the USSR" deals comprehensively with all the most important problems of the development of public health and medicine in the Soviet Union. The considerable increase in the funds assigned to the requirements of public health (over and above the control figures of the development of the national economy of the USSR in 1959-1965, a supplementary sum of 1800 million rubles has been allocated by decree for 1961-1965), provides the material basis for the rapid and general development of public health, including the further growth of medical science.

The decree of the Central Committee of the CPSU and the Council of Ministers of the USSR represents the concrete realization of the decision of the Twenty-First Congress of the Communist Party of the Soviet Union, to the effect that: "In the present stage of the formation of a communist society, science is becoming ever more important." The importance of science is concretely stated and repeatedly emphasized in the decree, especially with regard to the fulfillment of the enormous tasks confronting Soviet medicine. As the decree emphasizes, the perfect solution of practical problems necessitates that attention be concentrated on "the fundamental problems of the theory of medicine (physiology, biochemistry, ctyology, virology, and immunology)." The attention paid by the party and the government to the theory of medicine is emphasized by a special instruction to the Councils of Ministers of the union republics on the necessity for taking steps to strengthen the medico-biological divisions of the Academies of Sciences of the union republics.

In the light of the foregoing, great importance will be attached to the further increase of the activity of medical journals, among them the theoretical journals devoted to topical problems of modern medicine and biology. Our own journal, in particular, is confronted by new tasks, foremost among which is to ensure the speedy publication of original work in the different fields of experimental biology and medicine, which is up to the standard of present-day methods, and of theoretical and practical importance for medicine.

Under these circumstances, the journal is raising its requirements for work submitted from the point of view of its factual worth, and the accuracy of the documentation and presentation, and it will make its pages available first and foremost to the authors of articles in which problems of medical importance are boldly presented and solved in an original manner. The stricter selection of articles will permit the quicker publication of valuable and necessary work. Considerable attention will be paid to the popularization of modern and original methods of investigation, to the introduction of new experimental techniques, the use of various forms of apparatus borrowed from adjacent fields of science and the combination of experimental and clinical methods of investigation.

The carrying out of these and other measures will result in a further raising of the general and scientific standard of the journal in accordance with the new targets for Soviet medical and biological science at their present stage of development.

PHYSIOLOGY

CHANGES IN THE BIOELECTRICAL ACTIVITY IN VARIOUS AREAS OF THE CEREBRAL CORTEX IN DOGS, DURING THE FORMATION OF A TEMPORARY CONNECTION

V. N. Dumenko

Laboratory of Higher Nervous Activity (Head – Academician of AN Ukrainian SSR G. V. Fol'bort) of the A.A. Bogomolets Institute of Physiology (Director – Corresponding Member AN Ukrainian SSR A. F. Makarchenko), AN Ukrainian SSR, Kiev

(Presented by Active Member AMN SSSR V. V. Parin)
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In the extensive literature on electroencephalography there is comparatively little data on the interrelationships between individual cortical areas. Certain authors [1, 2, 10, 11] have reported the synchronous flow of α-waves in different areas, and have explained this by irradiation of activity. Having noted the synchronism of the waves of action potentials, however, these authors have failed to give an adequate explanation of the physiological meaning of the phenomenon.

We investigated this problem [3] in M. N. Livanov's laboratory [4, 6, 7], when we showed that during the formation of a temporary connection in a rabbit, there is an evident similarity in the fluctuations of the action potentials in the cortex of the corresponding analyzers.

It was felt to be of considerable interest to examine the changes in the interrelationships between the waves of action potentials in various areas of the cortex during the formation of a temporary connection in dogs.

METHOD

Experiments were carried out on three dogs in a soundproof, screened room, and were of long duration.

Using the method described by L. G. Trofimov and R. N. Lur'e [7, 8], 11 electrodes were inserted into the cranial bones of each animal. When unipolar leads were used to tap the potentials, the indifferent electrode was applied to the nasal bones of the animal, and when bipolar leads were used the distance between the electrodes did not exceed 3-4 mm.

The potentials were fed into amplifiers, assembled by a symmetrical resistance-capacitance coupling scheme, and recorded on a loop oscillograph.

RESULTS

When the bioelectrical activity was simultaneously recorded from two points of the cortex situated within the bounds of the visual or auditory analyzers, it could be seen that waves of action potentials develop at nearly identical times. Differences were observed in the amplitude and also in the magnitude and duration of the reaction to adequate stimulation (especially in the cortex of the auditory analyzer, but the background activity was synchronous (Fig. 1, a, 1).

On the other hand, when action potentials were recorded simultaneously from two points of the cortex, one of which was situated in the visual analyzer and the other in the auditory, hardly any resemblance was shown in the timing of the bioelectrical activity.

In the same way, waves of action potentials were asynchronous when taken from two points of the cortex of the motor analyzer (or two areas when bipolar leads were used) corresponding to the representation of the fore and hind limbs*, even when the distance between them was 5 mm. No resemblance could then be observed between the flow of action potentials, whether with unipolar or bipolar leads (Fig. 1, b).

In dog No. 1, for instance, a very clear rhythm (about 25 waves per second) was recorded in the area of representation of the forelimb, which disappeared on passive elevation of the left fore limb (see Fig. 1, b). An analogy may evidently be drawn between this rhythm and the Rolandic rhythm in man.

It is apparent from the same figure that in the area of representation of the hind limb the amplitude of the action potentials was increased in response to passive elevation of the paw. At the same time a clear synchronism was observed between the waves of potentials in two points situated either within the area of representation of the forelimb or that of the hind limb (Fig. 1, a, 2).

These observations, which were evidence of the fine differentiation of the nucleus of the motor analyzer, demonstrated the necessity for accurate designation of the areas from which leads are taken for recording the EEG from the motor area.

*The correct positioning of the electrodes was verified by electrical stimulation of areas of the cortex situated beneath the inserted electrodes.

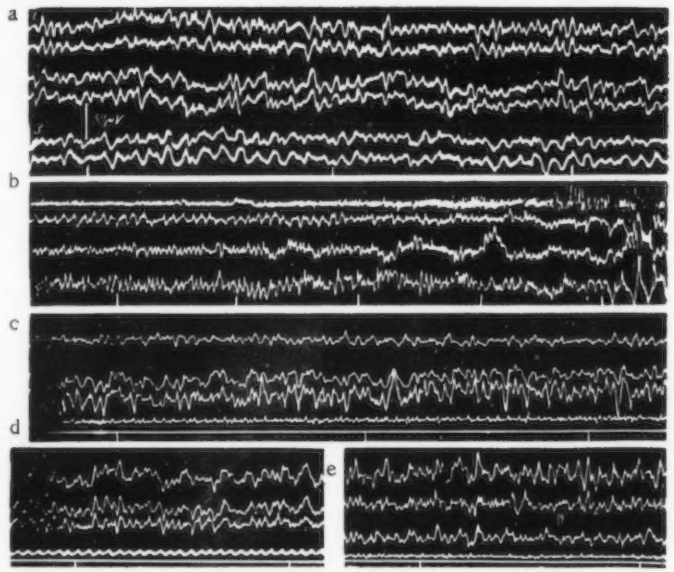

Fig. 1. Character of the interrelationships between the action potentials in the cortical ends of various analysors. a) Before formation of a conditioned connection (unipolar lead). From above down: 1) EEG from two points of the cortex in the area of the visual analysor; 2) the same, from the area of representaiton of the fore-limb; time marker (0.5 second); b) the same as in a (bipolar lead). From above down: Electromyogram (EMG) of the flexors of the fore limb; EEG of the cortex of the visual analysor; EEG from the area of representation of the hind limb; time marker (0.5 second). The arrow indicates the beginning of passive elevation of the limb; c) in response to a combination of a light and passive flexion of the forelimb (unipolar lead). From above down: EEG of the cortex of the visual analyzer; EEG taken from the area of representation of the hind limb; EEG from the area of representation of the fore limb; EMG; stimulation marker; time marker (0.5 second); d) the same as in c. From above down: EEG from one point in the area of representation of the forelimb; EEG from another point in the same area; EEG taken from the area of representation of the hind limb; EMG; stimulation marker; time marker (0.5 second); e) the same as in c and d. From above down: EEG from one point in the area of representation of the hind limb; EEG from another point in the same area; EEG of the visual analysor; EMG; stimulation marker; time marker (0.5 second).

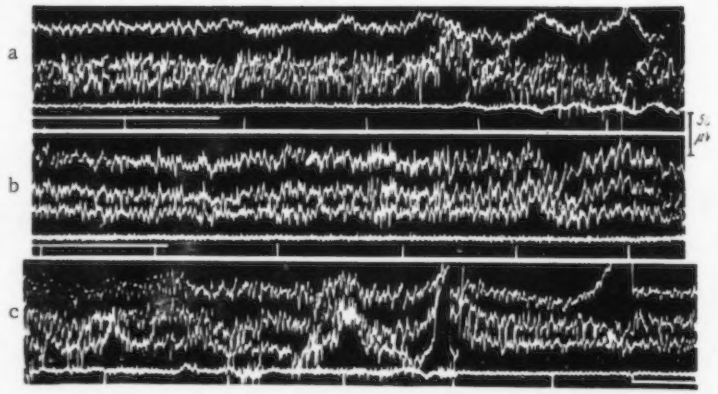

Fig. 2. Electrical activity of the nuclei of different analyzers during the time of action of a conditioned stimulus (unipolar lead). From above down: a) EEG from the area of the visual analyzer; EEG from one of the points of a "synchronous pair" in the area of representation of the hind limb (beneath the 8th electrode); the same, in the area of representation of the fore limb (beneath the 10th electrode); EMG stimulation marker; time marker (0.5 second); b and c) EEG from one point in the area of representation of the hind limb (beneath the 8th electrode); the same, in the area of representation of the fore limb (beneath the 10th electrode); EEG taken from the cortex of the visual analysor; EMG; stimulation marker; time marker (0.5 second) (c — to be read from right to left).

No regular rhythm was observed in two other dogs (Nos. 2 and 3), although the relationship between the electrical activity in the various areas of the cerebral cortex was the same as in the previous dog.

It was considered of interest to examine the changes in these relationships during the period of formation of a temporary connection. For this purpose a conditioned reflex was formed in dog No. 1 in response to a combination of the light of an electric lamp (5 seconds) and passive elevation of the left fore limb. Into the cranial bones of this dog were inserted 11 electrodes: 5 electrodes over the nucleus of the motor analyzer (the 8th and 9th—over the area of representation of the hind limb; the 10th and 11th — over that of the fore limb; the 7th over the area of representation of the hind limb in the opposite hemisphere); 2 electrodes (5th and 6th)—over the cortex of the auditory analyzer; 2 electrodes (3rd and 4th)—over the cortex of the visual analyzer; and 2 electrodes (1st and 2nd) were used as indifferent electrodes.

After the first [1-3] combinations, an evident change in the magnitude of the electrical reaction in the cortex of both the visual and the auditory analyzers was observed in response to the action of the light. No appreciable changes were observed in the interrelationships between the waves of action potentials in the nuclei of the different analyzers.

The increased reactivity observed in the first stages of the formation of a temporary connection was preserved, however, for only a very short time, and had disappeared by the 4th-5th combination. It was evidently brought about by an orientational reaction of the animal.

It can be seen from the electroencephalograms of this subsequent period that no change had taken place in the rhythm observed previously in the area of representation of the forelimb (see Fig. 1, b) in response to light; perceptible reactions in the area of representation of the hind limb and in the cortex of the visual analyzer were also absent.

Immediately after discontinuation of the sixth combination, the appearance of an obvious similarity between the bioelectrical waves in the areas of representation of the fore and hind limbs was observed for the first time. At the 8th combination this similarity was also maintained in the background activity, and it was further preserved during the combination and the after-action.

After two more experiments, the areas in which synchronism of the waves of action potentials was observed became concentrated. Synchronism was now observed only between two separate points of the cortex (see Fig. 1, c) one of which was situated in the area of representation of the forelimb (beneath the 10th electrode) and the second in that of the hind limb.

A pair of points with synchronous activity was thus formed in the nucleus of the motor analyzer. The similarity between the potentials of these points and of their pairs within the area of representation of the same limb was disturbed (compare Fig. 1, d and e with Fig. 1, a, 3). In addition, an obvious increase in reactivity was observed in these points of the cortex and also in the area of the visual analyzer in response to the action of the conditioned stimulus (Fig. 2, a).

These changes in the interrelationships between individual areas of the cortex in the areas of representation of the fore and hind limbs were evidently due to complex coordinational relationships, established in the nucleus of the motor analyzer when the animal was standing with its limb elevated. At this time, however, the cortex of the visual analyzer, to judge by the electrograms, was not yet connected with the cortex of the motor analyzer of the corresponding side.

At this stage synchronism was observed in the activity of the visual area and that of the area of representation of the hind limb on the opposite side. At the time of appearance of the conditioned reflex elevation of the limb, an obvious synchronism was found between the potentials in the nucleus of the visual analyzer and the "synchronous pair" of the nucleus of the motor analyzer (Fig. 2, b).

It is perfectly clear that in this case there are no grounds for speaking of manifestations of desynchronization. This is especially interesting because the combined stimuli were nonrhythmic and, as many authors have observed [5, 9], during the formation of conditioned reflexes to nonrhythmic stimuli changes of a desynchronizational character are found.

After the formation of a conditioned reflex to light, a defensive conditioned reflex to sound was formed.

A few combinations of a sound with electrical stimulation of the skin led to the disappearance of the selective synchronism, described above, in the activity of the two points in the cortex of the motor analyzer, and to the restoration of the original relationships (synchronism within the bounds of the area of representation of the fore and hind limbs).

Subsequently, during repetition of the combinations of the sound and the electrical stimulation of the skin, the synchronism between the action potentials within the area of representation of the fore limb became considerably weakened, and was preserved only in the area of representation of the hind limb in which the conditioned reflex was formed. At this time the cortex of the auditory analyzer gradually became involved in activity which was synchronous with the activity of the area of representation of the hind limb.

On reapplication of the combination of the light and elevation of the forelimb (after $1^1/_2$ months), the corresponding conditioned reflex appeared at the first trial; synchronism of the action potentials in the nuclei of the visual and motor analyzers was also restored (Fig. 2, c).

It must be emphasized that the synchronism of the bioelectrical activity, described above, was observed when the waves of potentials in each of the areas under comparison were polyrhythmic in character. Synchronization of rhythms could frequently arise without special preliminary procedures (especially by the use of rhyth-

mic stimulation). Similarity between action potentials in the form of irregular waves (especially if the areas of the cortex to be compared belonged to different analyzers) appeared only in response to the action of combinations.

These phenomena are of great interest, for they are evidence of selective connections between particular areas of the cortex.

It may evidently be suggested that the appearance of synchronism between waves of action potentials in particular areas of the cerebral cortex, which is found at a certain stage in the formation of a conditioned reflex, is one of the signs of the establishment of a temporary connection.

SUMMARY

Under the combined effect of two stimuli (nonrhythmic light and passive raising of the forelimb), a considerable change occurred in the character of the bioelectric activity in various cortical areas.

At first, these changes were limited to the nucleus of the motor analyzer (synchronic course of the waves of action potentials at two points of the cortex located in the areas of representation of the fore and hind limbs). Later, there appeared synchronous waves of action potentials between these two points and the nucleus of the visual analyzer. It may be suggested that the similarity in the waves of action potentials of various cortical portions points to the presence of selective connections between them and is one of the signs indicating establishment of a temporary connection.

LITERATURE CITED

[1] I. Beritov, Trudy Inst. Fiziol. im. Beritashvili I. Tbilisi 5, 193 (1943).

[2] I. Beritov and D. Gedevanishvili, Trudy Inst. Fiziol. im. Beritashvili I. Tbilisi 6, 279 (1945).

[3] V. N. Dumenko, Trudy Inst. Vysshei Nerv. Deyatel., Ser. Fiziol. 1, 335 (1955).

[4] I. N. Knipst, T. A. Korol'kova, et al., Proceedings of the Ninth Congress of Physiologists (Minsk, 1959), p. 238 [In Russian].

[5] A. Kogan and N. Nikolaeva, Proceedings of a Conference on the Electrophysiology of the Central Nervous System (Moscow, 1958) p. 65 [In Russian].

[6] M. N. Livanov, Transactions of the Fifteenth Conference on Problems of Higher Nervous Activity (Moscow-Leningrad, 1952), p. 248 [In Russian].

[7] M. N. Livanov, Proceedings of a Conference on the Electrophysiology of the Central Nervous System (Leningrad, 1957) p. 77 [In Russian].

[8] R. N. Lur'e and L. G. Trofimov, Fiziol. Zhur. SSSR 4, 348 (1956).

[9] A. I. Roitbak, Proceedings of a Conference on the Electrophysiology of the Central Nervous System (Moscow, 1958) p. 106 [In Russian].

[10] E. D. Adrian and K. Yamagiwa, Brain 58, 323 (1935).

[11] R. Cohn, J. Neurophysiol. 11, 31 (1948).

GENERAL AND LOCAL CHANGES IN THE ELECTROENCECPHALOGRAM DURING THE FORMATION OF CONDITIONED MOTOR REFLEXES IN MAN

Ts'u Chih-p'ing

Laboratory of Electrophysiology (Head -- Corresponding Member AMN SSSR Prof. V. S. Rusinov) of the N. N. Burdenko Research Institute of Neurosurgery (Director - Active Member AMN SSSR B. G. Egorov), AMN SSSR, Moscow

(Presented by Active Member AMN SSSR B. G. Egorov)

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The method of electroencephalography is being used on an ever widening scale in the investigation of higher nervous activity. Many researchers have shown that conditioned reflex changes in the α-rhythm may be obtained. The conditioned reflex depression of the α-rhythm obtained by a combination of sound and light has been studied by Durup and Fessard [9], Loomis, Harvey and Hobart [11], Jasper and Shagass [10], P. I. Shpil'berg [8], and others.

It has been shown that a conditioned reflex reorganization of the cortical rhythm can be obtained in man by the action of a rhythmic light stimulus [4, 6]. Changes in the electrical activity in the cortical divisions of the brain during the formation of conditioned motor reflexes have been studied in man by V. E. Maiorchik, V. S. Rusinov and G. D. Kuznetsova [3], I. A. Peimer and A. A. Fadeeva [7], T. M. Mokhova [5], H. Gastaut and his co-workers [1, 2] and others, by the method of verbal reinforcement or by preliminary instruction.

During the formation of delayed inhibition, slow waves were found to appear at the time of depression of the motor reflexes [3]. These authors came to the conclusion that these slow waves, including delta waves, were the electroencephalographic reflection of the change of the cortical cells into a state of inhibition.

In recent years researchers have paid increasing attention to the local reactions of the cortical rhythms, which differ from the general reaction—the blocking of the α-rhythm in the occipito-parieto-temporal region. In work by Gastaut, Roger, Dongier, et al. [1, 2], a conditioned reflex block of the rhythm in the motor area of the cortex of one hemisphere (the so-called Rolandic rhythm) was obtained during the formation of conditioned motor reactions, and an increase in the amplitude of the potentials in this area was obtained during the development of central inhibition.

The aim of the present study was to make a further investigation of the changes in the electrical activity of the cerebral cortex during the formation of conditioned motor reflexes in man. In the present communication we examine changes in the electroencephalogram (EEG), generally found in healthy human subjects and in patients with pronounced cerebral disorders.

METHOD

Investigations were carried out on 40 healthy human subjects and on 8 patients with sequelae of slight head injuries, in the phase of recovery and with no focal nor significant general cerebral changes, aged from 18 to 52 years. Conditioned motor reactions were developed by the method of verbal reinforcement (Ivanov-Smolenskii's method) to complex sound stimuli, consisting of three equal sound signals given at regular intervals. For differentiation, complex sound stimuli were used, also consisting of 3 signals: The first two signals were of the same tone as the conditioned positive signals, but the third was of a different tone. The sound signals were produced by means of a type 3G-2A sound generator or a photophonostimulator. During the investigation the subject sat on an armchair in a darkened, screened room.

In the course of formation of the conditioned motor reactions, tracings were made of the EEG and the electromyogram (EMG) from the right forearm. Bipolar EEG recordings were made on an 8-channel Kaiser ink-recording oscillograph or on a 15-channel Alvar ink-recording electroencephalograph. The electrodes for recording the EEG were situated chain fashion on the skull, from the frontal to the occipital regions, at a distance of 4 cm from each other and 2-3 cm from the midline. We were thus able to examine simultaneously the reactions in the EEG in different areas of the cortex. Before the formation of conditioned motor reactions the orientational reaction to sound was extinguished. The electrodes used for recording the EMG were in the form of round disks, 1 cm in diameter, which were glued to the right forearm with collodion. Altogether 62 investigations were made.

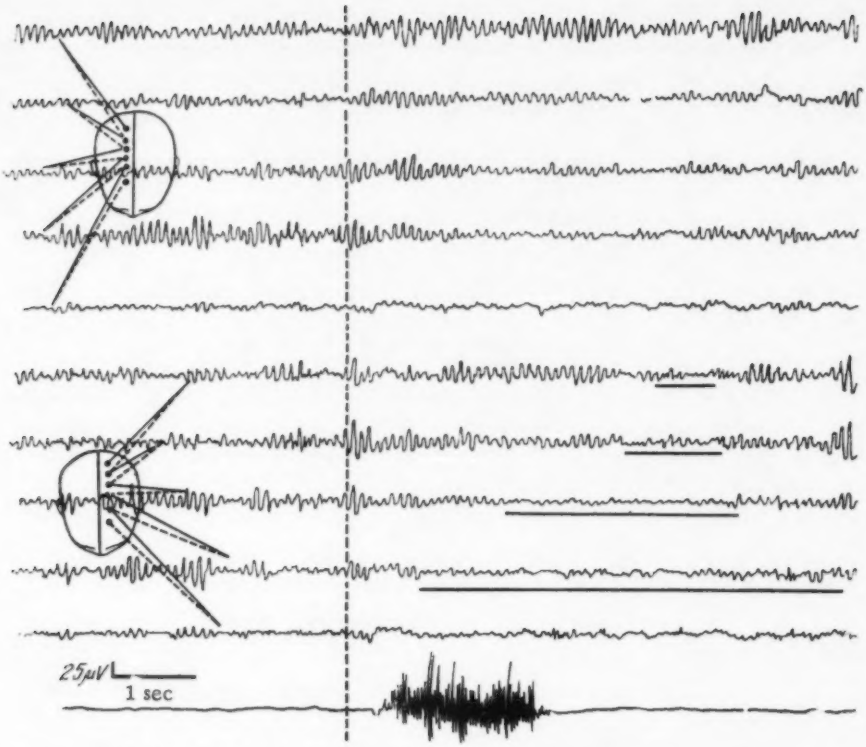

Fig. 1. EEG of the subject P. during an arbitrary movement. Lower line – EMG of the right forearm; the broken vertical line corresponds to the time of giving the instruction "squeeze"; the leads correspond to the illustration.

RESULTS

At the beginning of the investigation the light signal was given several times and the subject was then asked to clench the fingers of the right hand into a fist or to squeeze a rubber balloon. During the light stimulation we observed, like other authors, a general block of the α-rhythm in all areas; if the subject had a well-marked Rolandic rhythm, after the first application of the light an incomplete and transient depression of this rhythm was observed (about 0.2 second); after the repeated application of the light signal the Rolandic rhythm was not depressed but in some cases was even increased in amplitude.

During the first motor reactions two types of changes were observed in the EEG. In one group of subjects there was a general depression of the rhythms in all areas of the cerebral cortex, arising immediately after the word "squeeze." It may be thought that this general depression was caused by the influence of a nonspecific system in the brainstem. This depression of the rhythms subsequently had a tendency either to be concentrated in the motor area as the motor reactions were repeated, or to disappear. In the other group an obvious local reaction was observed, in the form of depression of the α-like rhythm in the motor of the left hemisphere, which began immediately after verbal reinforcement, before the development of the motor reaction (from the EMG).

It is interesting that in some cases the blocking of the rhythm was observed to spread from the motor to the posterior divisions of the brain, and depression of the motor area was still present after a considerable time (Fig. 1).

The reactions in the EEG to sound stimuli were the same in both groups of subjects. The first application of an indifferent sound stimulus is known to evoke general depression, which is extinguished after a few repetitions of the stimulus. When the reaction to each separate sound stimulus was extinguished, the use of complex sound stimuli, consisting of three equal sounds, caused no perceptible reaction in the EEG.

After extinction of the orientational reaction to the sound stimulus, this was used in conjunction with verbal reinforcement—the word "squeeze," given at the time of the 3rd sound signal of the group. After 3-9 combinations a conditioned motor reflex was formed.

In the course of formation of conditioned motor reactions in the first group of subjects, depression of the rhythms in all areas of the cerebral cortex was observed in response to the first combination of the complex stimulus including the word "squeeze." After repetition of the combination, concentration of the depression was observed in the motor area of the left hemisphere. This general depression may reflect a process of generalization of the excitation. In Fig. 2 we give as an example the

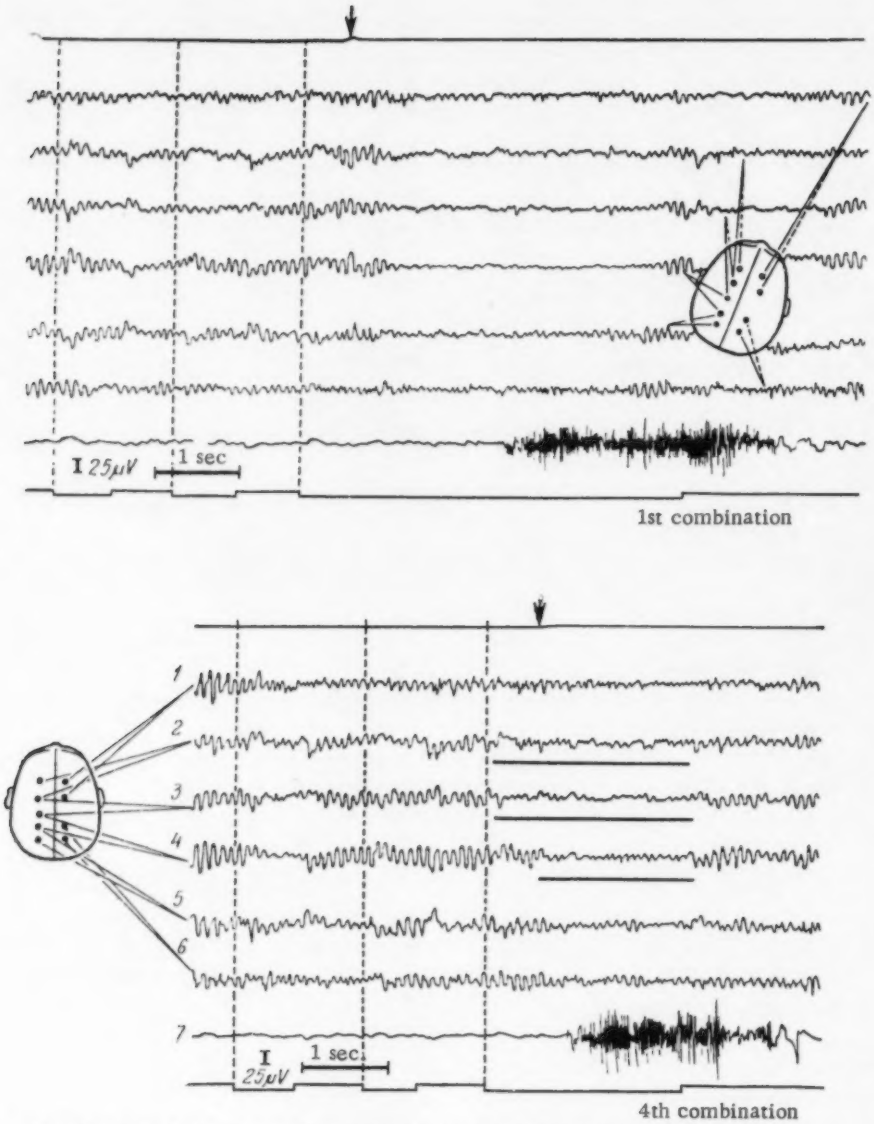

Fig. 2. EEG of the subject Ya. during formation of conditioned motor reflexes. a) 1st combination; b) 4th combination; top line – marker of verbal instruction (arrows). 2nd line from the bottom – EMG of the right forearm; depression of the bottom line – marker of sound stimulus; 3rd sound prolonged; leads corresponding to the diagram.

EEG of the subject Ya. In response to the first combination, depression of the α-rhythm is observed in all the leads after verbal reinforcement with the word "squeeze;" later, in response to the 4th combination, no general depression was recorded. It must be stated, however, that in the parietal motor areas (when leads were taken from a pair of electrodes situated in the motor and parietal areas and from another pair situated in the parietal and posterior parietal areas) the reaction of suppression of the rhythm was observed at the beginning of application of the 3rd conditioned signal of the group; in other areas a change in the action potentials was observed only during movement of the hand (from the EMG), and moreover the movement appeared after a shorter latent period, almost at once after the verbal reinforcement.

On presentation of the conditioned signals to the second group of subjects, only a local depression of the rhythms in the motor areas was observed. There was no generalized depression. During repetition of the conditioned stimulus, local depression was observed at the 3rd sound, before the fingers were clenched into a fist which may be evidence of the formation at that stage of a temporary connection. In some subjects a local reaction of the EEG to the first sound was recorded.

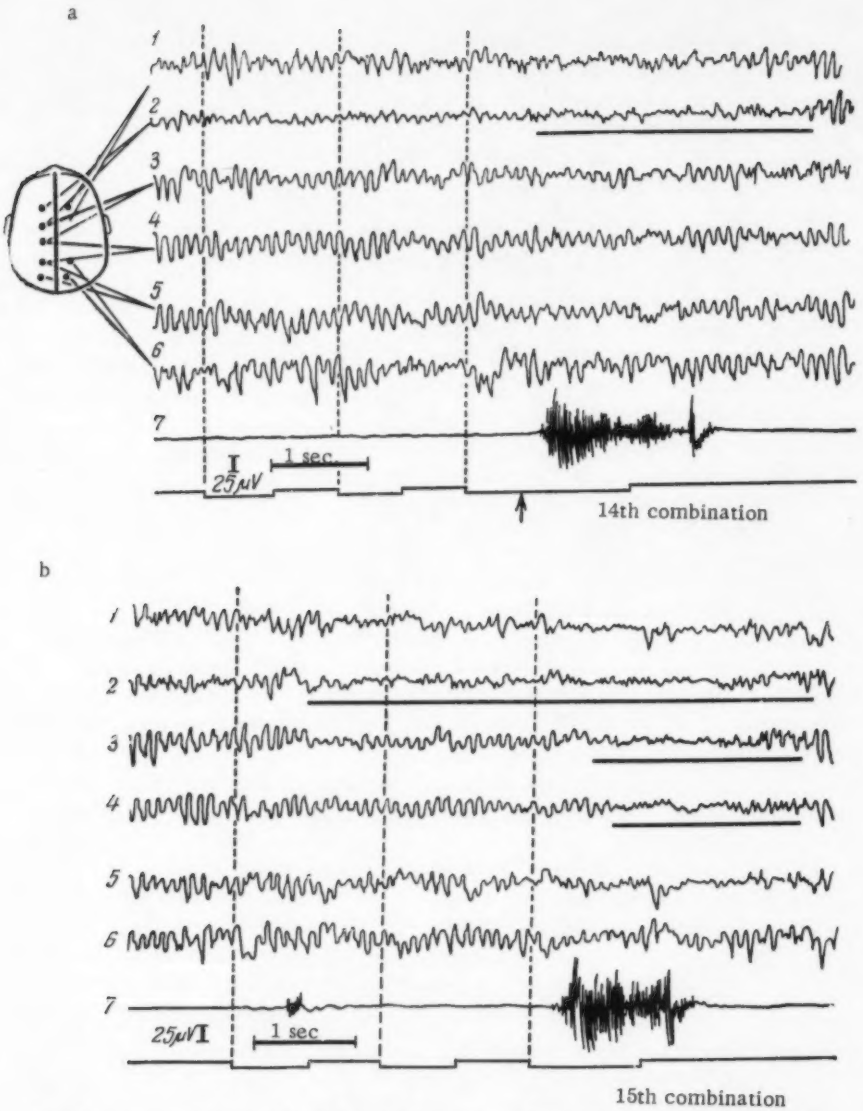

Fig. 3. Changes in the EEG during the formation of conditioned motor reactions in the subject P. a) 14th combination; local depression of the rhythm in the motor area on the left (2) was observed only in response to clenching the right hand; b) after the command, depression of the rhythm was observed at the first sound, lasting to the end of the movement; during the movement it also appeared in other leads; the leads correspond to the diagram; depressions of the bottom line – marker of the sound stimulus.

In cases in which there was prolonged inhibition of the conditioned motor reaction from the second signal system, i.e., the subjects responded by movement only after verbal reinforcement, the command "squeeze the fingers at the 3rd signal" was given during the investigation.

In response to the first application of the combined stimulus after the command, the character of the reaction in the EEG changed. Depression was now observed in response to the giving of the first signal of the group. In some subjects a general blocking of the rhythms in all areas was at first observed, but with the continuing application of conditioned signals, blocking was recorded only in the motor area of the left hemisphere. The blocking began at the first signal of the group, then the rhythm was restored, but the depression arose again at the beginning of the 3rd sound signal, before clenching of the fingers. In other subjects local changes, in the form of depression of the motor area only (Fig. 3), were observed in response to the first conditioned stimuli after the com-

mand. Depression began at the first signal and continued until the end of clenching the fingers into a fist. In response to subsequent conditioned signals, no depression of the rhythm was observed at the first signal of the group; it occurred only in response to the 3rd signal of the group.

After 15-20 combinations of the conditioned positive signal with verbal reinforcement the 3rd sound signal was omitted from the group. Under these circumstances, in some cases, a motor reaction to time was observed, expressed in the EMG in the form of waves of potentials of low amplitude. When the subjects were questioned after the investigation, they replied that they clenched their fingers "unarbitrarily." The EEG at the same time showed either depression of the α-rhythm in all areas or a local reaction in the sensomotor area.

In some observations the reaction to time was expressed in the EEG as a local, and not a general depression of the rhythms (in the sensomotor area), later spreading to the parietal and occipital regions. After depression, the α-rhythm was restored earlier in the occipital region, and then in the parietal region. Depression lasted longest of all in the motor areas, and its duration was shortest in the occipital area. On a multichannel recording of the EEG, depression was expressed in the form of a "funnel." This change was observed in response to the first and second times that the omission was practiced, and also at the beginning of formation of differentiation to the combined stimulus, or during transformation of the positive stimulus into an inhibitory stimulus, and vice versa. This phenomenon, not previously described in the electrophysiological literature, apparently reflects a process of irradiation and subsequent concentration in the cerebral cortex.

The following conclusions may be drawn from these investigations. During the formation of motor conditioned reflexes in man (by the verbal-motor method) in response to compound sound stimuli, consisting of three equal sound signals, general changes are observed in the action potentials of the EEG, in the form of depression of the α-rhythm, which has a tendency to become concentrated in the sensomotor area after the application of the 3rd signal of the group. During the first occasions on which the 3rd sound was omitted, at the moment after the 2nd sound corresponding to the time of the 3rd sound, spreading of the depression of the α-rhythm from the cortical end of the motor analyzer to the occipital region was observed; recovery of the rhythm took place in the opposite direction.

SUMMARY

The author studied the EEG changes in man during the formation of conditioned motor reflexes to complex sound stimuli.

The data obtained permit 3 types of reactions to be distinguished: 1) A generalized depression of the alpha-rhythm; 2) a local depression of the alpha-rhythm in the motor area of the "active" hemisphere, and 3) a depression in the form of a "funnel."

In the latter, the depression started from the motor area, and subsequently spread in the occipital direction. The recovery of the alpha-rhythm proceeded in the reverse direction. Depression in the motor area was retained the longest.

In the author's opinion, these changes in the electroencephalogram reflect an irradiating process in the cerebral cortex with its subsequent concentration.

LITERATURE CITED

[1] H. Gastaut, A. Joos, F. Morel, et al., Zhur. Vysshei Nerv. Deyatel. 7, 1, 25 (1957).

[2] H. Gastaut, A. Roger, S. Dongier, et al., Zhur. Vysshei Nerv. Deyatel. 7, 1, 185 (1957).

[3] V. E. Maiorchik, V. S. Rusinov, and G. D. Kuznetsova, The Physiological Basis of Neurosurgical Operations (Moscow, 1954), p. 48 [In Russian].

[4] V. E. Maiorchik and B. G. Spirin, Voprosy Neirokhirurg 3, 3 (1951).

[5] T. M. Mokhova, Zhur. Vysshei Nerv. Deyatel. 6, 2, 319 (1956).

[6] R. A. Pavlygina and V. S. Rusinov, cited by V. S. Rusinov, Paper at the Fourth International Electroencephalographic Congress in Brussels, 1957 (Moscow, 1957) [In Russian].

[7] I. A. Peimer and A. A. Fadeeva, Fiziol. Zhur. SSSR 42, 3, 319 (1956).

[8] P. I. Shpil'berg, Byull. Eksptl. Biol. i Med. 24, 6, 271 (1947).

[9] G. Durup and A. Fessard, cited by V. S. Rusinov, Zhur. Vysshei Nerv. Deyatel. 7, 6, 855 (1957).

[10] H. Jasper and C. Shagass, J. Exper. Psychol., 28, 373 (1941).

[11] A. L. Loomis, E. N. Hervey, and G. Hobart, J. Exper. Psych. 19, 249 (1936).

THE FUNCTIONAL SYMMETRY OF THE CORTICAL PART OF THE AUDITORY ANALYZER

V. M. Mosidze

Division of General Physiology (Head – Active Member AN SSSR I. S. Beritashvili) of the Institute of Physiology (Director – Prof. S. P. Narikashvili), Academy of Sciences of the Georgian SSR, Tbilisi

(Presented by Active Member AMN SSR P. S. Kupalov)

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Investigations by K. S. Abuladze [1] have shown that the auditory analyzer is characterized by strict functional symmetry.

He showed that after removal of the cortex of one hemisphere, conditioned salivation in response to a sound stimulus is abolished in the parotid gland on the ipsilateral side; salivation from the opposite gland is retained and undergoes no particular change.

We carried out experiments in the same area but, in contrast to K. S. Abuladze's experiments, in which ordinary sound stimulation was used as a conditional signal (when the dog hears a signal, supplied to the experimental room, with both ears), we employed isolated stimulation of either ear with a sound. This was done by means of a special apparatus which K. S. Abuladze suggested and which we have described [2].

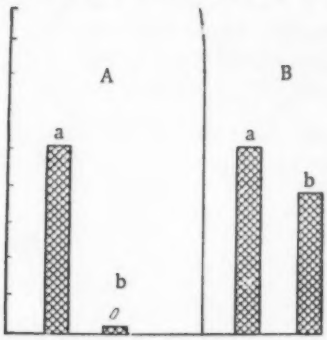

Fig. 1. Experimental results. The dog Sharik. A) Magnitude of the conditioned salivation from the left parotid gland after the isolated stimulation of the left ear with a sound before (a) and after (b) removal of the cortex of the right cerebral hemisphere; B) magnitude of the conditioned salivation from the same parotid gland after the isolated stimulation of the right ear with a sound before (a) and after (b) removal of the cortex of the right hemisphere. Along the ordinate axis – volume of saliva; columns – mean figures for 10 experiments.

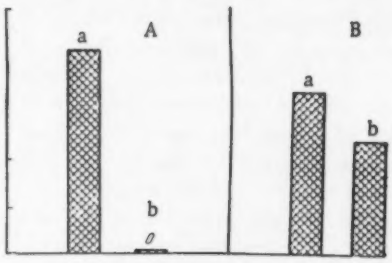

Fig. 2. Experimental results. The dog Kashtanka. A) Magnitude of the conditioned salivation from the right parotid gland in response to the isolated stimulation of the right ear with a sound before (a) and after (b) removal of the cortex of the left cerebral hemisphere; B) Magnitude of the conditioned salivation from the same gland after the isolated stimulation of the left ear with a sound before (a) and after (b) removal of the cortex of the left cerebral hemisphere. Along the ordinate axis – volume of saliva; columns – mean figures for 8 experiments.

METHOD

Experiments were carried out on 4 dogs, in a soundproof chamber, by the salivary conditioned reflex method. Chronic fistulae of the parotid salivary glands were created in the dogs by Glinskii's method, and conditioned auditory reflexes were established to the isolated stimulation of one ear with a sound (a tone or buzzer). In ad-

dition to the conditioned auditory reflexes, conditioned reflexes were formed in the dogs to a shaker and to visual stimulation (light). All the conditioned stimuli were reinforced by food—a fixed amount of powdered meat and biscuit. The volumes of the conditioned and unconditioned secretion of saliva were recorded in scale divisions by means of a Ganike-Kupalov pneumatic-hydraulic system, separately for the left and right parotid gland.

RESULTS

In the dogs Al'fa, Sharik, Kashtanka, and Usach, conditioned reflexes were formed to the isolated stimulation of the left and right ear with a sound. After this had been done, the cortex of one cerebral hemisphere was removed by operation. In all the experimental animals, extirpation of the cortex of the left cerebral hemisphere resulted in the complete disappearance of the salivary conditioned reflexes formed in response to the isolated stimulation of the right ear with a sound; conversely, after extirpation of the right cerebral cortex there was disappearance of the conditioned reflexes formed in response to the isolated stimulation of the left ear with a sound (Figs. 1 and 2). Experiments were performed at different times after the operation of the cortex of the cerebral hemisphere—from 10 days to 3 months. The conditioned auditory reflexes which disappeared after removal of the cerebral cortex were not restored in the course of 3-6 months.

In order to explain the results that were obtained, it must be considered that the greater part of the ascending fibers of the auditory nerve, after crossing in the lower portions of the brain, reach the cerebral cortex of the opposite hemisphere; the smaller part of these fibers reach the ipsilateral cortex. It is clear from our experiments that extirpation of the cortex of that hemisphere to which the conditioned excitation mainly passes (during isolated stimulation of the contralateral ear with a sound), causes the disappearance of the conditioned reflex on this side. The conditioned reflexes formed in response to the isolated stimulation of the opposite ear with a sound are preserved, since the conditioned excitation is addressed to the intact hemisphere. These experiments clearly show that the cortical part of the auditory analyzer consists of two symmetrical points, and that the removal of one symmetrical point of the cortex is not followed by a disturbance of the function of the other.

SUMMARY

Experiments were conducted on 4 dogs by the method of salivary conditioned reflexes. After elaboration of the background of the sound conditioned reflexes the cortex of one of the cerebral hemispheres was removed and the changes in these reflexes were subjected to study. On the basis of experimental data the author concludes that the sound analyzer is characterized by a strict functional symmetry. Removal of one symmetrical point of the cortex does not provoke any functional disturbance in the other.

LITERATURE CITED

[1] K. S. Abuladze, Byull. Eksptl. Biol. i Med. 41, 2, 14 (1956).*

[2] V. M. Mosidze, Data on Conditioned and Unconditioned Reflexes Before and After Partial Division of the Corpus Callosum in Dogs. Candidate's dissertation (Leningrad, 1955)[In Russian].

*Original Russian pagination. See C.B. translation.

REPRESENTATION OF THE INTERNAL ORCANS IN THE CEREBRAL CORTEX AND CEREBELLUM OF CATS AND DOGS COMMUNICATION IV. CORTICAL REPRESENTATION OF THE PELVIC AND PUDENDAL NERVES IN DOGS

K. M. Kulanda

Laboratory of General Physiology (Head – Active Member AMN SSSR V. N. Chernigovskii) of the Institute of Normal and Pathological Physiology (Director Active Member AMN SSSR V. N. Chernigovskii), AMN SSSR, Moscow

(Presented by Active Member AMN SSR V. N. Chernigovskii)

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In previous communications details were given concerning the representation of the pelvic [4] and pudendal [5] nerves in the cortex of the cerebral hemispheres in cats, and also findings related to the reception of information from the internal organs by the cortex [6].

In the present communication we describe facts obtained from experiments on dogs.

METHOD AND RESULTS

After it had been ascertained, in experiments on cats using the method of recording primary action potentials, that the zones of representation of the pelvic and pudendal nerves are situated within the confines of the specific projection fields of somatic sensation, viz. in the area of representation of afferents from the hind limb, it could be assumed that these zones would be similarly situated in dogs. Experiments carried out on 16 adult animals confirmed this*. It was found that in dogs, the zones of representation of these nerves were bilateral and were present in both fields I and II of general somatic sensation, where they were situated within the area of representation of the hind limb. As in cats, the zones of representation of the two nerves overlapped each other, and their foci of maximum activity (FMA) were situated in continuity with each other, although the size of the area occupied by each FMA was smaller than in cats. The FMA and the adjacent areas of the surface of the cortex did not coincide with the previous description [15, 18] of the cortical zones of representation of the splanchnic nerve.

In dogs, the first zone was situated in the anterior part of the presplenial gyrus. In Fig. 1 we show the results of an experiment in which the zones of cortical representation of the right pelvic nerve were defined. Tracing No. 1 illustrates the primary response in the FMA of zone I of the contralateral hemisphere. The latent period of this reaction was 12.9 millisec, and the amplitude (positive phase of the potential) was 100 μv. Besides the primary response, a series of secondary reactions is shown in Fig. 1 (tracings Nos. 2-10).

In this communication we shall confine ourselves to a very general description. Under light or moderately deep anesthesia, for instance, the secondary reactions were distinguished by their great profusion and variety. Their latent periods exceeded those of the primary reactions, and their extremely variable amplitude could be much greater than the amplitude of the primary response (see Fig. 1, tracing Nos. 1 and 10). Some of them arose in wide areas of the cortex, whereas others, on the contrary, were recorded within the limits of more localized areas, in immediate contact with the zones of specific projection. The secondary reactions were unstable towards anesthesia, and on increasing the depth of the latter they became more uniform, their latent period lengthened sharply and their "resistance" to repeated stimulation, i.e., their ability to develop in response to each individual stimulus, fell considerably. Tracings A and B must also be examined (see Fig. 1). Tracing A shows the primary response recorded in the same experiment in the contralateral zone II, during stimulation of the right pelvic nerve. Tracing B illustrates the action potential recorded in the ipsilateral zone I of the pelvic nerve during stimulation of that nerve. Attention is drawn to the smaller amplitude and longer latent period (14.8 millisec; 72 μv) of this ipsilateral response by comparison with the contralateral (see Fig. 1, tracing No. 1).

The second zone of cortical representation of the pelvic and pudendal nerves is situated in dogs in the middle part of the anterior ectosylvian gyrus.

* The method of these experiments is described in detail in Communication I [4].

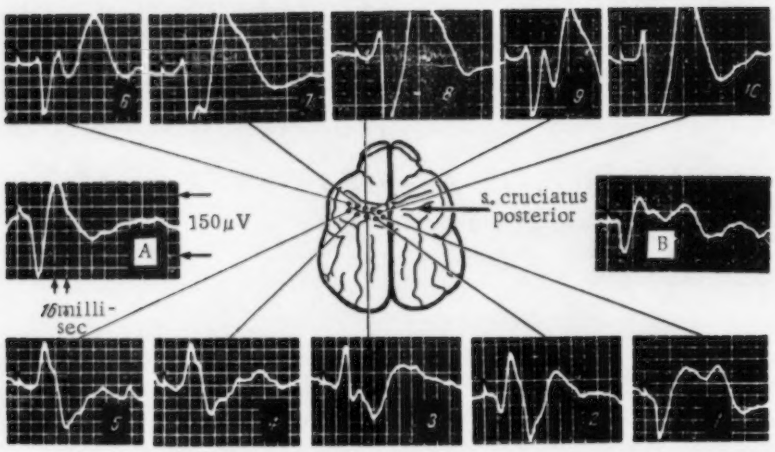

Fig. 1. Representation of the pelvic nerve in the cerebral cortex of a dog. Experiment on January 24, 1957. Anesthesia – chloralose. The right pelvic nerve was stimulated. Amplitude of stimulating impulse – 3.5 v; duration – 0.2 millisec. 1) Primary response in contralateral zone I; A) primary response in contralateral zone II; B) primary response – in ipsilateral zone I. The scale of amplification and time were the same for all the tracings (see text).

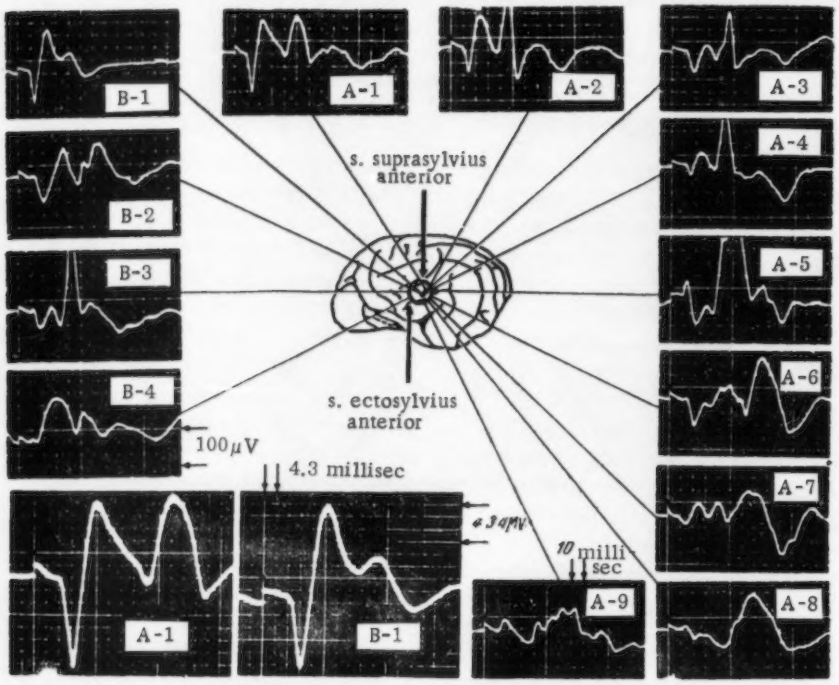

Fig. 2. Comparison between the representation of the pelvic (tracings A, 1-9) and pudendal (tracings B, 1-4) nerves in the contralateral zone II of the cerebral cortex of a dog. Experiment on February 18, 1957. Moderately deep anesthesia with a nembutal– chloralose mixture. Diplacin (a curare-like drug). Artificial respiration. Amplitude of stimulating impulse – 6 v; duration – 0.2 millisec. Scale of amplification and time for the enlarged tracings A–1 and B–1 given separately (see B–1), and for all the remaining tracings (see A–9 and B— 4).

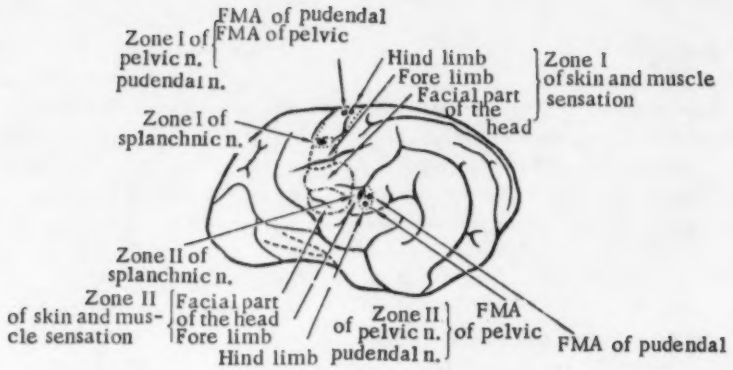
FMA of pudendal
Zone I of FMA of pelvic
pelvic n.
pudendal n.
Hind limb
Fore limb
Facial part of the head
Zone I of skin and muscle sensation
Zone I of splanchnic n.
Zone II of splanchnic n.
Zone II of skin and muscle sensation
Facial part of the head
Fore limb
Hind limb
Zone II of pelvic n. pudendal n.
FMA of pelvic
FMA of pudendal

Fig. 3. Diagram of the situation of the zones of representation of the pelvic and pudendal nerves in the cerebral cortex. The black circles designate the foci of maximum activity (FMA), i.e. those areas of the surface of the cortex from which primary potentials with the greatest amplitude and shortest latent period were recorded.

In Fig. 2 are shown the results of an experiment in which the representation of the two nerves was determined concurrently. The tracings from A-1 to A-9 are the bioelectrical reactions arising in the contralateral zone II in response to stimulation of the central end of the right pelvic nerve with a current of 6 v. The tracings from B-1 to B-4 are the bioelectrical reactions arising in this zone in response to stimulation, of the same intensity, of the central end of the right pudendal nerve. A-1 and B-1 are tracings of the primary responses in the FMA of the corresponding nerves. Tracings A-2 and B-2 were taken after a slight displacement of the electrode from the FMA of one of the nerves towards the FMA of the other nerve. The tracings from A-3 to A-9, B-3, and B-4 were taken within the zone outlined by a circle on the diagram of the brain.

Without dwelling on the analysis of the secondary reactions, we may take note of certain peculiarities of the primary responses. A-1 had a shorter latent period (9.3 millisec) than B-1 (10.9 millisec). Although the combined amplitudes of the positive and negative phases of the variations of potential in the two responses were equal, the amplitude of the positive phase in A-1 was greater than in B-1.

This superiority of the activity of the pelvic nerve over the pudendal was characteristic, and we observed it in both zones I and II of their joint representation in the cerebral cortex of dogs.

It has already been mentioned above that each nerve has its independent FMA, but that usually these areas of greatest activity are situated with each other; the experiment described illustrates this. We have mentioned in an earlier paper [4] the influence of the depth of anesthesia on the dimensions of the zones of representation and on the responses recorded therein. All that has been said on this subject in relation to experiments on cats is fully applicable to dogs also. The only difference observed was that, in dogs under deep anesthesia, the ratio between the area of the two zones to the whole surface area of the cortex was smaller than in cats. This is in agreement with observations [17] showing that in animals at a higher level in the phylogenetic scale, the relative size of the surface of the cortex occupied by the primary projection zones has become smaller on account of the greater development of the "associative" fields. The zones of representation of the nerves which we were investigating are shown in Fig. 3, in which the FMAs are indicated by black circles.

When the results obtained from the experiments on cats and dogs are assessed, the question must be asked—what is the reason for this arrangement of the zones of cortical representation of the visceral nerves, and what is their functional importance?

We consider that the zones of cortical representation of the nerves under study, which are component parts of the internal analyzer, may be regarded as, in the words of I. P. Pavlov, "locations of primary processes," which first receive the signals arriving from the periphery. Immediately afterwards, and parallel with this event, there occurs that cycle of processes which transforms the cortex into "a grandiose complex of positively and negatively excited points, closely and thoroughly intermingled with each other" [10].

These "locations of primary processes" in the cortex are areas within the bounds of which the specific projection systems terminate.† We mean these areas of the

† By specific projection system of an analyzer, we mean the paths conducting excitation from its peripheral end to its cortical end through the smallest number of serially connected neurones.

In contrast to this, by nonspecific projection systems, we mean paths which conduct excitation from the peripheral ends of different analyzers to the territory of the cortex in general through the multineuronal networks of structures in the brainstem.

cortex whenever we speak of "zones of cortical representation" of particular afferent nerves.

Attention is likewise drawn to the localization of the zones of representation of the visceral nerves (including the zones of representation of the splanchnic nerve) within the limits of those areas of the cortex in which are represented afferent nerves carrying impulses from the skin, muscles, joints and tendons (see Fig. 3). This localization is evidently not accidental, but it is the reflection, in the higher divisions of the central nervous system, of the close associations which have long been known to exist between the visceral afferent systems and the skeletal musculature.

In recent decades the problem of somato-visceral relationships has once again attracted the attention of many workers [3, 8, 9, 11, 13, 14]. V. N. Chernigovskii [13], for instance, showed that stimulation of the sinus and depressor nerves affected the locomotor centers of the cortex, facilitating the irradiation of excitation, as a result of which new muscle elements were involved in the contraction. He later [14] found that direct stimulation of the receptor fields of internal organs may also affect the skeletal musculature.

The presence of a definite functional connection between somatic and visceral structures was also suggested by the experiments of M. R. Mogendovich [9], who showed that stimulation of internal organs may change the chronaxie of the skeletal musculature.

That there is a very close connection between the somatic and visceral afferent system was also shown by the findings of Amassian [15, 16], that the zones of representation of the splanchnic nerve coincided with the zones of representation of skin and muscle sensation in the corresponding areas of the body. These experiments shed light on the activity of the mechanisms by means of which a close association is achieved between somatic and vegetative functions, and it is, moreover, evident that such mechanisms are situated not only in the cerebral cortex but also at other levels of the central nervous system. Everyday practice provides many examples in which the activity of the internal organs is bound up in the closest manner with the activity of the skeletal musculature. It will suffice to recall the acts of vomiting, parturition, micturition, and defecation, and the tension of the abdominal muscles arising immediately after an acute lesion affecting certain internal organs. It is well known, however, that the phenomena observed in the coordinating activity of the systems mentioned above require the direct participation of cortical mechanisms in by no means every case. In S. I. Frankshtein's experiments [12], for instance, mechanical compression of the urinary bladder, or distension of the organ from within, led to inhibition of the extensor rigidity of the hind limbs of a decerebrate cat, i.e., had a direct effect on the skeletal musculature. It is obvious that such an interrelationship did not require the participation of the cerebral cortex.

On the other hand, the facts discovered by E. A. Airapet'yants, N. Vasilevskaya, and A. Perel'man [1], who succeeded in transforming stimulation of the mucous membrane of the intestine into the conditioned signal of a defensive reaction, cannot be explained without accepting the direct participation of cortical mechanisms. We believe that it is this purpose which is served by the zones of representation of afferent nerves which have been found in the cortex.

In other words, if we accept E. A. Asratyan's [2] point of view, the zones of cortical representation of the sensory nerves may be regarded as the functional and morphological basis of the cortical representation of the unconditioned reflex.

The fact that the zones of representation of the nerves which we studied, like the zone of representation of the splanchnic nerve, are situated in that part of the cortex in which are represented the afferent systems of the corresponding somatic structures, is evidently a reflection of the segmental principle of the innervation of the internal organs.

The coincidence between the zones of cortical representation of the visceral and somatic sensory nerves provides the basis for a new approach to the mechanism of production of hyperalgesia in diseases of the internal organs. It is obvious that the existence of Zakhar'in-Head zones may be explained not only by an axon reflex, or a reflex effected at the spinal cord level, but more probably by the fact that afferent impulses from the affected organ reach the same cortical zones (and even the same cortical cells) as impulses from the skin, muscles, joints, and tendons. The correctness of such a hypothesis is further confirmed by the fact that hyperalgesia is often manifested in the muscles, bones, and other structures, as well as in the skin.

It has previously been mentioned that stimulation of any visceral receptor field can lead to the appearance of both primary and secondary reactions in the cerebral cortex. This does not mean, however, that all impulses arising in the internal organs must inevitably reach the cerebral cortex and be incorporated in the sphere of perception and consciousness. We have obtained results which testify to the fact that impulses reaching the central nervous system from somatic structures are capable of completely blocking conduction in the afferent pathways leading from the internal organs [7]. If, for example, soon after a series of stimuli applied to the sciatic nerve, stimulation is transferred to the pelvic nerve, no primary responses can be found in the zone of its cortical representation; they are completely abolished by the previous stimulation of the somatic nerve. Does not this phenomenon explain the well known fact that we call signals arriving from internal organs sensations only when they reach a considerable intensity? That is, they announce either injury to the corresponding visceral structures, or a functional state which is such as to require urgent interference of a voluntary character.

As an example of this, we may cite the signals reaching the central nervous system from the urinary bladder which we are unable to detect, of which we remain unaware, until they become so intensive that no muscular movements are capable of preventing the passage of impulses to the higher divisions of the nervous system. With such a functional state of the organ, the necessity for voluntary interference is obvious.

Such are the general outlines of the functional importance of the zones of cortical representation of the visceral nerves.

SUMMARY

By recording primary responses, 2 zones of representation of the afferent fibers of the pelvic and pudendal nerves were found in the cortex of each cerebral hemisphere in dogs. The zones are bilateral, with their localization in fields I and II of the general somatic sensation, within the area of representation of the hind limb. The zones of representation of the pelvic and pudendal nerves overlap each other. However, each nerve has its own focus of maximal activity (FMA) which does not coincide with the FMA of another nerve.

In dogs, zone I of the cortical representation of the nerves under study is located on the anterior part of the presplenial gyrus and zone II on the medial portion of the anterior ectosylvian gyrus.

LITERATURE CITED

[1] E. Airepet'yants, N. Vasilevskaya, and A. Perel'man, Doklady Akad. Nauk SSSR 30, 3, 248 (1941).

[2] E. A. Asratyan, Physiology of the Central Nervous System (Moscow, 1953) [In Russian].

[3] A. M. Grinshtein, Pathways and Centers of the Nervous System (Moscow, 1946) [In Russian].

[4] K. M. Kullanda, Byull. Eksptl. Biol. i Med. 5, 3 (1957).*

[5] K. M. Kullanda, Byull. Eksptl. Biol. i Med. 1, 8 (1960).*

[6] K. M. Kullanda, Byull. Eksptl. Biol. i Med. 6, 3 (1957).*

[7] K. M. Kullanda, Proceedings of a Conference on Problems of the Electrophysiology of the Central Nervous System (Leningrad, 1957), p. 70 [In Russian].

[8] O. S. Merkulova, Byull. Eksptl. Biol. i Med. 27, 6, 419 (1949).

[9] M. R. Mogendovich, Sensitivity of the Internal Organs (Interoception) and the Chronaxie of the Skeletal Musculature (Leningrad, 1941) [In Russian].

[10] I. P. Pavlov, Complete Collected Works 3, 2, 11 (Moscow-Leningrad, 1951) [In Russian].

[11] G. T. Semenova, Byull. Eksptl. Biol. i Med. 5, 3 (1958).*

[12] S. I. Frankshtein, Byull. Eksptl. Biol. i Med. 10-11, 18 (1944).

[13] V. N. Chernigovskii, Fiziol. Zhur. SSSR 20, 261 (1936).

[14] V. N. Chernigovskii, Fiziol. Zhur. SSSR 33, 5, 657 (1947).

[15] V. E. Amassian, J. Neurophysiol. 14, 435 (1951).

[16] V. E. Amassian, Res. Publ. Ass. Nerv. Ment. Dis. 50, 371 (1952).

[17] P. Buser, J. Physiol. 49, 589 (Paris, 1957).

[18] C. B. B. Downman, J. Physiol. 113, 434 (1951).

*Original Russian pagination. See C.B. translation.

CHANGES IN THE LABILITY OF SMOOTH MUSCLE DURING STIMULATION OF THE SYMPATHETIC AND PARASYMPATHETIC NERVES

R. S. Orlov and Kh. S. Khamitov

Department of Physiology of the I. P. Pavlov First Leningrad Medical Institute and the Department of Physiology (Head – Corresponding Member AMN SSSR Prof. A. V. Kibyakov), Kazan Medical Institute

(Presented by Active Member AMN SSSR V. V. Parin)

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The antagonism between the actions of the sympathetic and parasympathetic nerves of smooth muscle structures is a well known fact. In some cases the sympathetic nerve causes contraction of a smooth muscle (for example, the retractor penis muscle of the dog, the nictitating membrane of the cat), and in others it causes relaxation (for example, the musculature of the bronchi, the gastrointestinal tract, and so on). A similar dualism is observed in the action of the parasympathetic nerve. For a long time the classical concept of a pure antagonism between the sympathetic and parasympathetic systems has been inadequate for the explanation of the variety of their mutual relationships. The nerves of one system may be either inhibitory or stimulatory, depending on the region which they innervate, and on the state of the tissue on which they act. In certain doses, the action of both adrenalin and acetylcholine may be mutually potentiating [1, 5, 9, 10].

In experiments carried out on a ring of smooth muscle from the taenia coli of the guinea pig, Bulbring [7, 8] obtained results which showed a connection between stretching and the frequency of the action currents and the membrane potential of the smooth muscle fiber. He found that the effect of mediators was similar to the electrotonic influence of two direct current poles. By the action of acetylcholine or the cathode, the frequency of the action potentials is increased and the membrane potential falls; conversely, by the action of adrenalin or the anode, a rise in the membrane potential and a decrease in the frequency of action currents are observed. Thus, when the interval of time between two action currents is lengthened (under the influence of adrenalin or of stimulation of the sympathetic nerve), relaxation of the smooth muscle of the taenia coli takes place, and conversely, when the action currents are more frequent (as a result of the action of acetylcholine or of stimulation of the parasympathetic nerve), every small increment of tension following after every action current undergoes summation with others of the same kind, thereby creating a general contraction.

These findings suggest that the action of antagonistic nerves usually consists essentially of a change in the functional state, a change in the interval between the excitations in the smooth muscle cell, i.e., in other words to a change in the lability of the cell. We therefore undertook to investigate the action of the sympathetic and parasympathetic nerves on smooth muscle, and to determine the lability of the latter.

METHOD

Experiments were carried out on 27 dogs under morphine-urethane anesthesia. The test object used was the retractor penis muscle of the dog, innervated by both sympathetic (sympathetic trunk) and parasympathetic (pelvic nerve) nerve fibers. Stimulation of the sympathetic nerves causes contraction of the muscle, and stimulation of the parasympathetic nerves—relaxation. Immersible electrodes were used for the nerves, and platinum needle electrodes for stimulation of the muscle. We determined the limits of the optimal frequency and also the frequencies which led to pessimal relaxation during direct stimulation of the muscle. These values were determined before and after stimulation of the sympathetic and parasympathetic nerves, and also after giving the animal an intravenous injection of either 1% cocaine (after $1^1/_2$-2 hours) or adrenalin (after 30-40 minutes) in a dilution of 1:1000, or eserine in a dilution of 1:10,000 (after 30-40 minutes). In the experiments in which we recorded the bioelectrical activity, the muscle was attached under isometric conditions. The currents of action in response to direct stimulation of the muscle were taken by nonpolarizing electrodes to the input of a type UIPP-2 constant current amplifier with a coefficient of amplification of 50,000 and recorded on the tape of an MPO-2 8-loop oscillograph. A reduction in the magnitude of the loops of current during

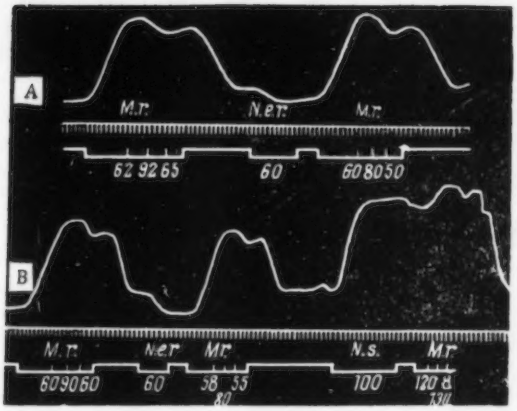

Fig. 1. Optimum and pessimum of the frequency of stimulation before and after stimulation of the sympathetic and parasympathetic nerves. Significance of the curves (from above down): myogram, time marker (5 seconds), stimulation marker. Figures — frequency of stimuli per second. M.r. — direct stimulation of the muscle; N.e.r.— stimulation of the parasympathetic nerve; Ns. — stimulation of the sympathetic nerve. A and B — tracings in two experiments.

direct stimulation was achieved by the use of a separating transformer. The index of lability was the highest frequency of the action currents and the level of their amplitude. The magnitude of the resting currents was shown in millivolts on the scale of the amplifier.

RESULTS

In the experiments using the myographic method of recording, obvious shifts were observed in the limits of the optimal and pessimal frequencies of direct stimulation of the muscle immediately after stimulation of the nerves. In all cases during stimulation of the pelvic nerve (parasympathetic nerve), the limits of the optimum and pessimum were displaced towards the lower frequencies. As may be seen from Fig. 1, A, before stimulation of the pelvic nerve, the optimal contraction developed at a frequency of stimulation of 62 imp/sec. After stimulation of the pelvic nerve, the limits of the optimum were shifted to 60 imp/sec, and the pessimum now developed at a frequency of 80 imp/sec. A different picture was observed after preliminary stimulation of the sympathetic nerve. The limits of the optimal and pessimal frequencies of stimulation in this case were displaced towards the higher frequencies. In Fig. 1, B, we show the myogram from one of the experiments. We can see that before stimulation of the nerves, the limits of the optimum and pessimum were respectively: 60 imp/sec—optimum, and 90 imp/sec—pessimum; after stimulation of the pelvic nerve these limits fell: to 58 imp/sec—optimum, and 80 imp/sec—pessimum, but after stimulation of the sympathetic nerve they were displaced towards the higher frequencies: the optimum amounted to 120 imp/sec and the pessimum developed at a frequency of stimulation of 130 imp/sec.

Investigation of the bioelectrical potentials showed that the myographically recorded changes in the functional properties of the smooth muscle were reflected in the bioelectrical picture. The magnitude of the resting current, as measured in 10 experiments, was on the average 16-25 mv. During stimulation of the sympathetic nerve a decrease was observed in the value of the resting current, by 5-7 mv. Stimulation of the parasympathetic nerve most often led to an increase of 3-5 mv in the resting current. During direct stimulation of the muscle, biphasic potentials of regular shape were recorded, which is explained by the parallel course of the muscle fibers.

Action currents of great amplitude were found at a frequency of stimulation of 50-70 imp/sec. Increasing the frequency of stimulation to 80-90 imp/sec caused a sharp fall in the amplitude of the action currents and disturbed the correlation between the waves of action currents and the frequency of the applied stimulation (Fig. 2, A). After stimulation of the pelvic nerve, the amplitude of the action currents of the muscle fell; with an increase in the frequency of stimulation above 60 imp/sec, a pessimal reaction was observed (Fig. 2, B).

Stimulation of the sympathetic nerve increased the functional properties of the smooth muscle, as shown by an increase in the amplitude of the action currents and by the widening of the limits of the optimum. A fall in the amplitude of the action currents took place only at a frequency of stimulation of 100-120 imp/sec (Fig. 2, C).

During the investigation of the action of stimulation of the sympathetic and parasympathetic nerves on the functional mobility of smooth muscle, in a series of experiments we injected drugs and, on this background investigated the functional state of the smooth muscle. Injection of 2-3 ml of a 1% solution of cocaine, or of 0.2 ml of 1:1000 adrenalin solution usually led to displacement of the limits of the optimal and pessimal frequencies of stimulation towards the higher frequencies, soon after the injection. Eserine, when injected in a dose of 2-3 ml (concentration of 1:10,000), caused a displacement of the values of optimal and pessimal stimulation towards the lower frequencies. This effect was usually apparent 20-30 minutes from the moment of injection of the eserine.

The experimental results thus show that excitation of both the sympathetic and parasympathetic nerves influences a very important index of the functional state of a muscle—its lability. In this particular case, stimulation of the sympathetic nerve brings about a displacement of the limits of the optimum and pessimum towards the higher frequencies, an increase in the amplitude and frequency of the action currents and a fall in the resting current. Excitation of the parasympathetic nerve causes the opposite effect: Displacement of the limits of the

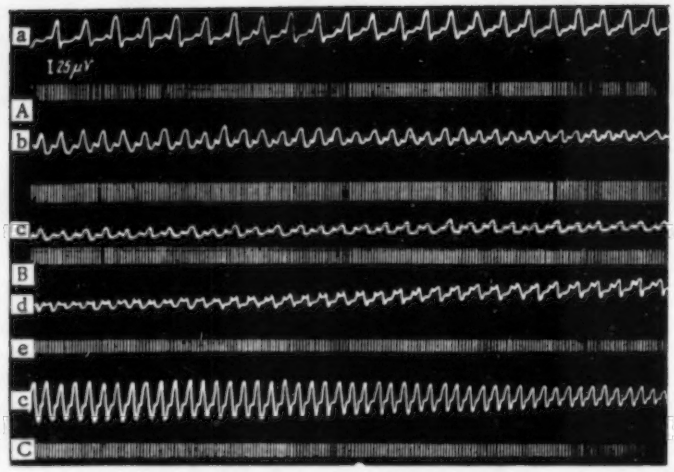

Fig. 2. Action currents of smooth muscle during direct stimulation of the muscle before and after preliminary stimulation of the sympathetic and parasympathetic nerves. A – before stimulation of the nerves: a-frequency of stimulation 50-60 imp/sec, b-frequency of stimulation 80-90 imp/sec; B – after stimulation of the parasympathetic nerve: c-frequency of stimulation 60 imp/sec, d-frequency of stimulation 70 imp/sec; C – after stimulation of the sympathetic nerve; e-frequency of stimulation 100-120 imp/sec. Time-marker – 0.02 second. Calibration in millivolts.

optimum and pessimum toward the lower frequencies, a reduction in the amplitude and maximum frequency of the action potentials in response to direct stimulation of the smooth muscle and an increase in the resting current. Substances having a sympathotropic or parasympathotropic character act in the same way as excitation of the nerves. Thus the sympathetic nerve, causing contraction of the retractor penis muscle, and the parasympathetic nerve which causes it to relax, affect the functional mobility of smooth muscle.

Smooth muscle is known to preserve its tone in preparations deprived of nerves and ganglion cells [11] and also during the action of ganglion-blocking drugs, as shown by its tonic contraction and by spontaneous discharges of action potentials. The tonic state of the smooth muscle is probably due to changes in muscle metabolism. The level of the muscle metabolism of the body, however, is to a large extent determined by nervous influences, and mainly by the mediators of nervous influence – sympathin and acetylcholine [3,4]. Work by A. V. Kibyakov and N. G. Bohdanov [2] showed that firstly, after division of nerves, and in particular the sympathetic nerve, the tonic state of smooth muscle is not disturbed; secondly, degeneration of divided sympathetic nerves at a certain period completely abolishes its tone; and thirdly, interference with the synthesis of sympathin leads to a sharp fall in the tone of smooth muscle. It may be assumed that the action of nerves, besides their trigger action, is directed towards changing the tonic state of smooth muscle which, evidently, is effected through the constant influence of mediators, which are also liberated when no excitation of the nerves is taking place. Excitation of the sympathetic nerve by means of the mediator, sympathin, increases the lability of the smooth muscle, gives rise to an increase in the amplitude and frequency of the action potentials. Excitation of the parasympathetic nerve, in this case through the medium of acetylcholine, lowers the lability of the smooth muscle, as shown by the decrease in the amplitude and frequency of the action potentials during direct stimulation of the muscle.

The "antagonism" between the sympathetic and parasympathetic innervation of smooth muscle in all probability consists essentially of their action on the functional properties of the muscle and, in particular, on its lability.

SUMMARY

The effect of sympathetic and parasympathetic nerves of a smooth muscle – the retractor penis – was investigated. Stimulation of the sympathetic nerve extends the limits of the optimal and pessimal frequency of stimulation towards greater frequencies, the action current frequency and amplitude increase, while the value of the resting current decreases.

On stimulation of the parasympathetic nerve, the action current frequencies become decreased, their amplitude declines and the resting current rises.

A suggestion is made that the "antagonism" of the sympathetic and parasympathetic innervation of the smooth muscle apparently is due to the action exerted on the functional properties of the muscle, particularly on its lability.

LITERATURE CITED

[1] A. V. Kibyakov, The Nature of the Regulating Influence of the Sympathetic Nervous System (Kazan', 1950) [In Russian].

[2] A. V. Kibyakov and N. G. Bogdanov, Fiziol. Zhur. SSSR 43, 12, 1170 (1957).

[3] Kh. S. Khamitov, Some Mechanisms of the Nervous Reflex Regulation of Smooth Muscle. Candidate's dissertation (Kazan', 1953) [In Russian].

[4] Kh. S. Khamitov, Fiziol. Zhur. SSSR 44, 5, 485 (1958).

[5] V. S. Sheveleva, Izvest. Akad. Nauk SSSR, Ser. Biol. 2, 94 (1956).

[6] E. Bulbring, J. Physiol. 103, 55 (1944).

[7] E. Bulbring, J. Physiol. 128, 200 (1955).

[8] E. Bulbring, Brit. M. Bull. 13, 172 (1957).

[9] E. Bulbring and J. H. Burn, J. Physiol. 101, 289 (1942).

[10] W. B. Cannon and A. Rosenblueth, Am. J. Physiol. 112, 268 (1935).

[11] D. H. Evans and H. O. Schild, Nature 180, 341 (1957).

THE DEMARCATIONAL POTENTIAL DIFFERENCE AS A CHARACTERISTIC OF THE CHANGING STATE OF POLARIZATION OF SKELETAL MUSCLES AT DIFFERENT AGE PERIODS

I. I. Gokhblit and I. A. Kornienko

Laboratory of Age Physiology and Pathology (Head — Prof. I. A. Arshavskii) of the Institute of Normal and Pathological Physiology (Director — Active Member AMN SSSR V. N. Chernigovskii), AMN SSSR, Moscow

(Presented by Active Member AMN SSSR V. V. Parin)

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It is now established [1-8] that at different stages of ontogenesis the nerves of mammals, and the skeletal muscles which they supply, are characterized by low lability, a low level of excitation, a long constant of the time of development of excitation (i.e., a long chronaxie), a long constant of accommodation (λ), a long refractory phase (absolute and relative), and a low coefficient of resistance, i.e., a low accomodational resistance to the action of an alternating stimulus. In newborn rats, on the 3rd-4th day of life, and in rabbits and cats in the intrauterine period, excitation in a nerve develops not at the cathode (in accordance with Pfluger's law of polarity), but at the anode. At early stages in the life of mammals, the nerve conductor responds to a single stimulus not by a single action current but by a diminishing discharge of impulses. These findings are evidence that at early age periods the neuromuscular apparatus is characterized by signs typifying a catelectrotonic state of the tissue. In the process of ontogenesis, and especially at critical stages in the individual development of the animal, the neuromuscular apparatus acquires signs typifying an anelectrotonic state of the tissue. This is shown by a gradual increase in the lability, level of excitation, shortening of the constant of accommodation and of the refractory phase (absolute and relative), an increase in the coefficient of resistance, and an increased ability to respond (albeit in the region of the cathode) to a single stimulus by a single action current [1-8]. These characteristic features of the main parameters of the neuromuscular apparatus suggest that their differences, depending on the age of the animal, can be explained only if it is accepted that they are due to variations in the properties of the membranes of nerve and muscle tissue.

In the present investigation an attempt has been made to demonstrate by more direct methods the possibility of an association between the polarization properties of the neuromuscular apparatus and the physiological characteristics listed above.

One direct index of the state and level of polarization of nerve and muscle tissue is the magnitude of the resting current. If, in the process of ontogenesis the change in the properties of the neuromuscular apparatus is in fact due to changes in the membrane, it is evident that this must be reflected above all in the magnitude of the demarcation potential difference. We made our starting point the membrane theory of action potentials, according to which the damaged part of the tissue is depolarized and becomes electrically negative in relation to the undamaged surface of the tissue. The magnitude of the potential difference thus created depends on the degree of polarization of the undamaged surface of the tissue.

METHOD

Experiments were carried out on unanesthetized rabbits of different ages, starting from the first days of life. The gastrocnemius muscle was carefully dissected and a transverse incision made in its distal end. The nerve was left intact during this procedure. The core of one nonpolarizing electrode (Zn, $ZnSO_4$, NaCl), moistened with Ringer's solution, was placed in contact with the incised surface of the muscle, and the core of another electrode was applied to the middle of the undamaged surface of the muscle. The demarcation potential difference was recorded by means of a mirror galvanometer ($1 : 10^{-9}$ A), included in an ordinary potentiometric compensation circuit, at room temperature 18-20°. We made 78 observations.

RESULTS

From the results obtained, the rabbits investigated could be divided into 3 age groups. The first group comprised rabbits aged from one to 10-12 days old (40 experiments), the second group—rabbits aged from 12 days to $1^1/_2$ months (18 experiments) and the third group—adult rabbits (20 experiments).

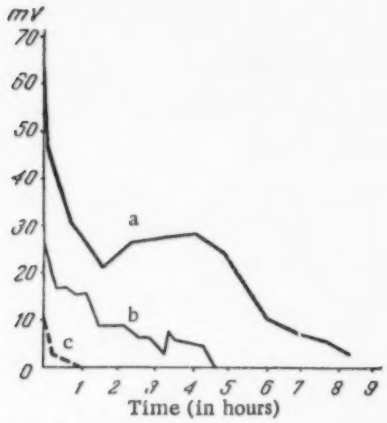

Fig. 1. Changes in the magnitude and duration of the demarcation potential difference in rabbits. a) In the third age group; b) in the second; c) in the first group (see text).

In each experiment we recorded the magnitude of the demarcation potential difference arising after infliction of the transverse incision, and the changes taking place in this magnitude as it gradually disappeared with time.

In the rabbits of the third group, the magnitude of the demarcation potential difference varied between limits of 30 to 100 mv. The mean value in the majority of experiments was 45-65 mv. In two experiments a potential difference of 25 mv was observed, and in another two experiments, one of 110-140 mv.

In the rabbits of the first age group the recorded magnitude of the demarcation potential difference varied between limits of 3 and 18.5 mv. The mean value in the majority of experiments was 6-11 mv. In 2 rabbits in the period of maturation, values of 22.5 and 33 mv were recorded.

In the rabbits of the second age group, the demarcation potential difference varied between limits of 15 and 46 mv. The mean value of the potential difference was 25-35 mv.

The length of time taken for the demarcation potential difference to disappear varied in the rabbits of the third age group between limits of 3 and $8^1/_2$ hours, and in the majority of experiments it was 6-8 hours. In rabbits under 10-12 days old (the first age group) the time taken for the demarcation potential difference to disappear varied between limits of 20 minutes and $2^1/_2$ hours. The mean time in the majority of experiments was 50-80 minutes. In only one rabbit (12 days), in which the magnitude of the demarcation potential difference was 33 mv the time taken for it to disappear was $3^1/_2$ hours. In rabbits aged from 12 days to $1-1^1/_2$ months (second age group), the time taken for disappearance of the demarcation potential difference varied between limits of 2 and $6^1/_2$ hours. The mean value in the majority of experiments was 3-4 hours. In one experiment the time taken for the disappearance of the potential difference was 1 hour.

In Fig. 1 are shown typical curves of the changes in magnitude and duration of the demarcation potential difference in rabbits in the various age periods.

The top curve (a) shows the dying away of the potential difference in adult rabbits. A sharp fall in the potential difference is seen during the first hour, and then for a short time the value of the potential difference is stabilized, after which it gradually tends to zero. The bottom curve (c) shows the disappearance of the demarcation potential difference in the rabbits of the first age group. This can be seen to occur within the first hour. The middle curve (b) represents the rabbits of the second age group, in which, after an initial, fairly sharp fall, the demarcation potential difference gradually tends towards zero.

Can it be considered that the difference which we found in the time taken for the demarcation potential differences to disappear may be used as an additional index of the state of muscle tissue, which is determined by age?

Dubois-Raymond first directed attention to the fact that the potential difference found in the isolated muscle of a frog between normal and injured areas fell by roughly 75% during the first 30 minutes. The progressive fall in the demarcation potential at the injured end of a nerve was subsequently studied by Engelman [11]. In order to account for this phenomenon, Gerard [12] put forward the hypothesis that at the line of demarcation from the site of injury a pseudomembrane is formed from the coagulating plasma. After the newly formed membrane has become completely polarized, the demarcation current disappears. Gerard attributed essential importance here to oxidative processes, as the factor producing and maintaining the electrical polarization of the limiting membranes. It must be pointed out that, in Gerard's hypothesis, repolarization of the regenerated membrane is looked upon as a passive process. In order to provide an explanation for the phenomenon described, we may turn to experiments which we carried out on the myocardium of the ventricle of the frog's heart.

If the anterior surface of the wall of the frog's ventricle is subjected to local cathode alteration through nonpolarizing electrodes, the following phenomenon can be observed at a suitable potential difference. The depolarization arising immediately beneath the cathode relaxes the altered area of the myocardium and excludes it from systolic activity. Immediately beneath the electrode, and in a shape corresponding strictly to it, an area of relaxed, red myocardium is formed, with the appearance of local diastole or asystole, lasting during systole of the ventricle. Alongside the electrode the state of polarization of the myocardium is increased. Closer to the periphery of the ventricle a state of depolarization is also induced perielectrotonically, which also arises beneath the electrode [2].

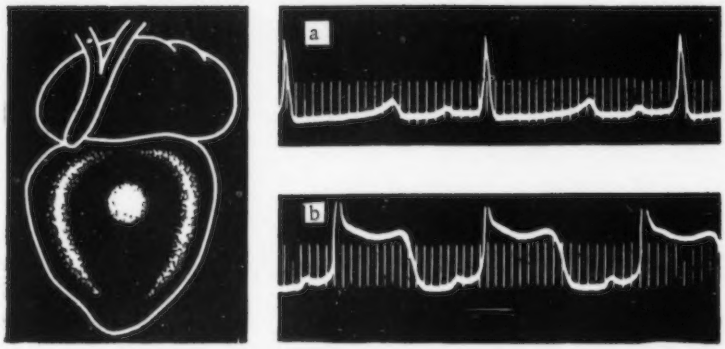

Fig. 2. Changes in the electrogram of the frog's heart. a) Before alteration; b) immediately after cathode alteration. On the left — scheme of distribution of contrast changes on the heart muscle after cathode alteration.

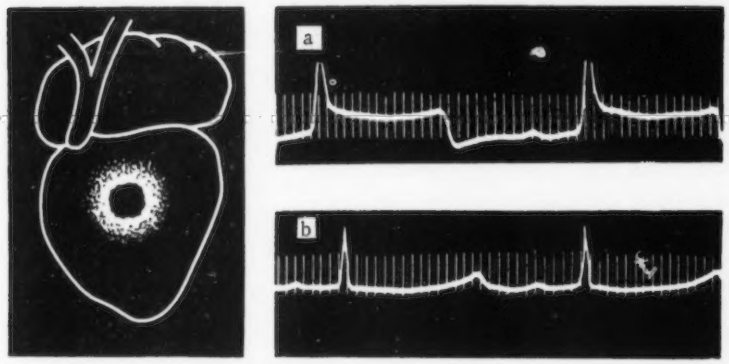

Fig. 3. Gradual disappearance of the monophasic potentials in the electrogram of a frog's heart during the formation of a necrotic zone at the site of cathode alteration (a,b). On the left — diagram of the contrast changes observed during the development of necrosis.

The asymmetry caused by local cathode alteration leads to the conversion of the biphasic action current of the ventricle into a monophasic current (Fig. 2).

If, during local cathode alteration of the ventricle, the current is increased and, in particular, its action is prolonged, then under these circumstances it can be seen how the depolarization formed beneath the electrode gradually changes into an irreversible necrosis. The relaxed, red myocardium beneath the electrode disappears and, in its place, is formed a white necrotic focus, around which appears a border of red, relaxed myocardium, not previously seen here, in apparent asystole. This depolarized border of relaxed myocardium, as it moves across to the periphery of the focus of alteration, naturally continues to maintain the monophasic character of the action potential of the ventricle of the heart.

When the border of depolarization arises at the periphery it remains for 30-60 minutes, after which it disappears completely, whereas the focus of necrosis is preserved. With the disappearance of the border of depolarization, its monophasic character is gradually lost and is replaced by the normal biphasic action potential (Fig. 3).

The gradual disappearance of the monophasic potential is none other than the expression of the gradual disappearance of the demarcation current. The disappearance of the monophasic and renewal of the biphasic action potential are evidence of the depolarization of the membrane which is regenerating at the border with the focus of necrosis. Regeneration of the membrane must be regarded as a process characterizing an active reaction which takes the form of an increase in metabolism and polarization in contiguous points of the tissue at the border with the parabiotic focus of injury.

It may be thought that the phenomena demonstrated in the ventricle of the frog's heart also take place in skeletal muscle after injury caused by a transverse incision.

The time taken for disappearance of the demarcation action potentials thus represents the time during which

the area of muscle, injured by a transverse incision, passes into a state of irreversible necrosis. Differences occurring in the time taken for the demarcation potential differences to disappear are a reflection of differences in the time taken for the altered muscle tissue to pass into a state of irreversible necrosis. This length of time varies in accordance with the age of the animal. It is natural to suppose that a difference so striking as that which characterizes the duration of the change into a state of irreversible necrosis is, in all probability, an additional and frequent expression of low functional resistance or of low accommodational resistance to the alteration in early stages, and of the gradual increase in the functional resistance in the process of ontogenesis.

If the magnitude of the demarcation current is taken as an index of the degree of polarization, it must be concluded that the level of polarization of muscle tissue is very low in early stages and increases each time a change is made from one stage of individual development to the next.

A study of the resistance of the gastrocnemius muscle, with its connections to nerve centers intact, to an audiofrequency (5000 cps) or high frequency (1,000,000 cps) current enabled the coefficient of polarization to be deduced. In adult rabbits the total apparent resistance was 1500 ohms and the coefficient of polarization about 4. In rabbits aged under 8-10 days, the total apparent resistance to an audiofrequency current was about 600 ohms and the coefficient of polarization 1.5. In rabbits aged from 10 days to 1 month, the total apparent resistance was 750 ohms and the coefficient of polarization 2.

It has already been stated that, in early stages of ontogenesis, the neuromuscular apparatus is characterized by the typical state of a catelectrotonic syndrome. In the light of the results obtained, this state may be explained by a lowering of the level of polarization of the muscle and also, evidently, of the nerve tissue. The acquisition by the neuromuscular apparatus, in the process of ontogenesis, of the typical properties of an anelectrotonic syndrome may be explained by a gradual increase in the level of polarization.

In the muscles of the frog and rabbit, a relationship has been found [9, 10] between the demarcation potential and the time taken for its disappearance from subordinating influences from the nerve centers.

It may be postulated that the differences which we found in the level of polarization in accordance with the age of an animal are due to changes in the course of ontogenesis in the subordinating or trophic influences from the nerve centers on the muscle tissue. The transformation of polarized membrane structures in the muscle tissue, brought about by the influence of the nerve centers, not only leads to an increase in its lability but also makes it possible for potential energy to develop in the muscle on an ever-increasing scale, while in a state of low resistance.

SUMMARY

Experiments were carried out on rabbits of various ages. The authors investigated the value of the demarcation potential difference in the gastrocnemius muscle and the time interval until it vanishes. In adult rabbits the average value of the demarcation potential difference was 45-65 mv. The time interval until it disappeared equalled 6-8 hours. In rabbits under 10-12 days old this average value amounted to 6-11 mv, with the average time interval until its complete disappearance equalling 50-80 minutes. In rabbits aged from 12 days to 1-1½ months these two figures were 25-35 mv and 3-4 hours.

The above data serve as additional characteristics of the changes occurring in the functional condition of the neuromuscular apparatus at various age periods.

LITERATURE CITED

[1] I. A. Arshavskii, Uspekhi Sovremennoi Biol. 32, 1, 18 (1951).

[2] I. A. Arshavskii and O. Kurmaev, Fiziol. Zhur. SSSR 18, 4, 592 (1935).

[3] I. A. Arshavskii and V. M. Kushnarev, Problems of Experimental Biology and Medicine 1, p. 48 (Moscow, 1951) [In Russian].

[4] I. A. Arshavskii and A. A. Oganisyan, Byull. Eksptl. Biol. i Med. 13, 3-4, 79 (1942).

[5] I. A. Arshavskii and V. D. Rozanova, Fiziol. Zhur. SSSR 26, 6, 629-640 (1939).

[6] Z. M. Esipovich, The Functional Resistance of the Peripheral Nervous System at Various Age Periods. Author's abstract of candidate's dissertation (Moscow, 1954) [In Russian].

[7] A. A. Oganisyan, Fiziol. Zhur. SSSR 34, 1, 87 (1948).

[8] V. D. Rozanova, Arkh. Biol. Nauk SSSR 46, 1, 123 (1937); Fiziol. Zhur. SSSR 25, 4, 391, 403 (1938); 3, 346 (1941).

[9] M. N. Farfel', Byull. Eksptl. Biol. i Med. 24, 3, 183-189 (1947).

[10] L. G. Khrolinskii, Fiziol. Zhur. SSSR 40, 4, 472 (1954).

[11] T. W. Engelman, Arch. ges. Physiol. 15, 116 (1877).

[12] R. W. Gerard, Am. J. Physiol. 93, 498-541 (1930).

THE ACTION OF PITUITRIN ON THE WORK OF THE GASTRIC GLANDS

A. S. Yampol'skii

Department of Normal Physiology (Head—Prof. S. S. Serebrennikov) of the Ivanov Medical Institute (Director—Docent Ya. M. Romanov)

(Presented by Active Member AMN SSSR V. V. Parin)

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Preparations of the posterior lobe of the hypophysis and, in particular, pituitrin, are widely used in clinical practice. Pituitrin is indicated in uterine inertia during labor, postoperative and postpartum retention of urine, uterine hemorrhage, diabetes insipidus, paralytic ileus, lowered blood pressure, and so on. In this connection the problem arises of the action of pituitrin on the body as a whole and, in particular, on the digestion in the stomach, as one of practical as well as theoretical interest.

The action of preparations of the posterior lobe of the pituitary on the secretory function of the stomach has been studied by many authors, but their findings are very contradictory. Several workers, for instance [16, 17, 19, 20], after injection of preparations of the posterior lobe of the hypophysis, observed an increase in the volume of gastirc juice and also in the free and total acidity. The majority of authors [1,2,4,5,6,7,8,10,14,18, and others], however, have observed inhibition of gastric secretion after the action of preparations of the posterior lobe of the hypophysis, and also a decrease in the acidity and digestive power of the juice. Certain authors [9 and others] were unable to detect any changes in the gastric secretion as a result of the action of preparations of the posterior lobe of the hypophysis.

Bearing in mind that in the majority of these investigations, particular attention was directed towards the changes in the volume of the gastric juice, and that changes in the composition of the gastric juice may be of no less importance in the pathology of digestion, we deci ded to study the qualitative changes arising in the gastric juice under the influence of pituitrin.

METHOD

Experiments were carried out on dogs. Of the 3 animals taking part in the experiments, two had isolated gastric pouches of the Heidenhain type (the dogs Rex and Raketa) and one, a Pavlov pouch as modified by G. M. Shpug (the dog Lyutik). The experiments were all performed in the same room. The dogs were kept on roughly identical conditions of diet.

Pituitrin P (produced at the Moscow factory for endocrine preparations, activity 3 i.u./ml) was injected subcutaneously and intravenously in a dose of 0.1 ml/kg body weight. In the majority of experiments the pituitrin was injected immediately after feeding the dog on meat (in the latent period of secretion), and in 2 experiments, on the dog Raket, pituitrin was injected intravenously one hour after a meal, i.e. at the height of secretion.

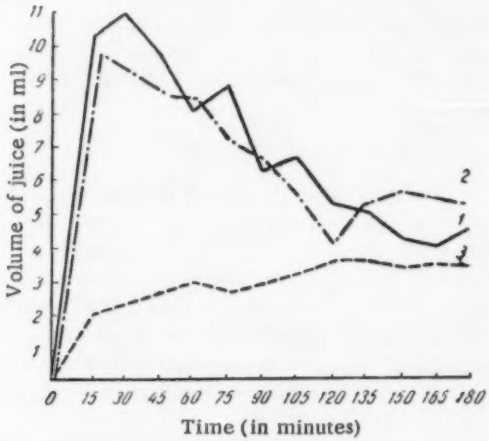

Fig. 1. Changes in the volume of gastric juice after the subcutaneous and intravenous (during the latent period) injection of pituitrin. 1) Control experiment; 2) subcutaneous injection of pituitrin; 3) intravenous injection of pituitrin.

Excitation of the gastric glands was caused by feeding 200 g of raw meat in the course of 5 minutes. Observations were made on the secretion of juice for 3 hours. In each hourly sample of juice we determined the protease and lipase activity, the free, combined and total hydrochloric acid contents, the total acidity, the specific gravity, and the content of dry residue, organic and inorganic substances.

The protease activity was determined by Mett's method. Ths lipase activity was estimated by the vol-

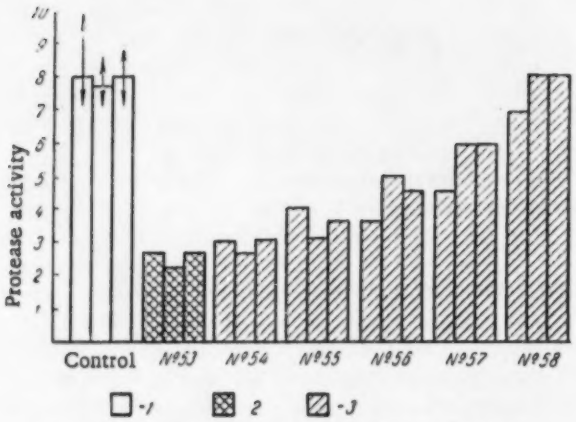

Fig. 2. Changes in the protease activity (in mm, by Mett's method) in the dog Rex after intravenous injection of pituitrin (during the latent period). 1) Mean results of control experiments; 2) protease activity on the day of injection of pituitrin; 3) protease activity on the days after injection.

ume of N/10 alkali used in the titration of the fatty acids formed in milk under the action of the lipase of the gastric juice. The free, combined and total hydrochloric acid contents and the total acidity were determined in the same sample by the generally accepted methods. In all, 92 experiments were performed on the three dogs.

RESULTS

After the subcutaneous and intravenous injection of pituitrin, the latent period of secretion was lengthened. Whereas in the dog Lyutik, for instance, this period normally lasted 10-18 minutes, after the subcutaneous injection of pituitrin it was extended to 35 minutes, and after intravenous injection—to 45-55 minutes. Lengthening of the latent period of secretion evidently suggests that pituitrin has a depressing action of the level of excitation of the gastric glands.

After the subcutaneous injection of pituitrin, the volume of gastric juice decreased slightly in all the experiments (Fig. 1), and corresponded to the lower limit of the variations in the control experiments. No more appreciable fall in gastric secretion could be observed. After the intravenous injection of pituitrin, however, during the latent period of secretion, we always observed a considerable decrease in the volume of gastric juice (to 1/2 or 1/4). If the pituitrin was injected at the height of secretion, then in the hour following injection, the volume of gastric juice fell sharply and amounted to 30-35% of the mean values in the control experiments. A fall in the volume of gastric juice, caused by the action of pituitrin, was also observed in the 1-2 days following its intravenous injection.

When we studied the changes in the enzymic activity of the gastric juice under the influence of pituitrin, in all the experiments we found a significant fall in the protease activity of the gastric juice, after both the subcutaneous and intravenous injection of pituitrin, regardless of the time of injection. Whereas after the subcutaneous injection of pituitrin, the protease activity fell on the average by 35-40%, however, after intravenous injection the preparation caused an even greater decrease in the protease activity, which amounted to only 20-25% of the average values of the control experiments. The intravenous injection of pituitrin resulted not only in a sharper but also in a more prolonged fall in the protease activity than after subcutaneous injection (Fig. 2). Whereas pituitrin, when injected subcutaneously, caused a decrease in the protease activity for 2-3 days, when injected intravenously in the same dose, it had the effect of depressing the protease activity for 5-6 days.

Pituitrin had a somewhat different effect on the lipase activity of the gastric juice (Fig. 3). The subcutaneous injection of this preparation caused hardly any changes in the activity of the lipase, if the fact that on

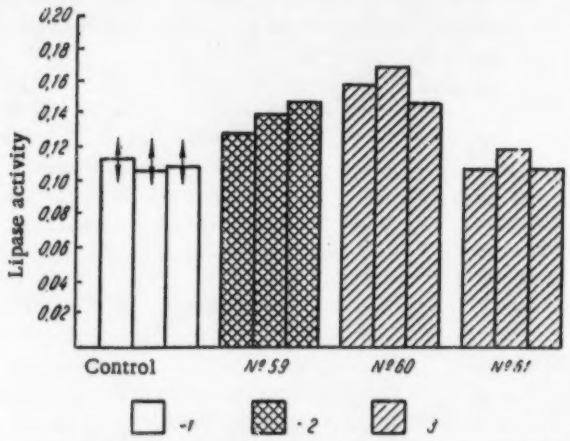

Fig. 3. Changes in the lipase activity (in ml 0.1/N NaOH) after the intravenous injection of pituitrin (during the latent period). 1) Mean results of control experiments; 2) lipase activity on the day of injection of pituitrin; 3) lipase activity on the days after the injection.

the day after injection the lipase activity rose (by 15-25%) is disregarded. After injection of pituitrin intravenously during the latent period of secretion the lipase activity on the day of the injection was either unchanged or slightly increased. On the next day we always observed a considerable increase in the activity of the lipase (by 40-60%), and in 2 experiments only, on the 3rd day, the lipase activity exceeded the upper limit of the variations of the control results; in the remaining experiments, on the 3rd day after injection of pituitrin no increase in the lipase activity was observed. The intravenous injection of pituitrin at the height of secretion produced a sharp increase (2-2 1/2 times) in the lipase activity in the 2nd hour of the experiment; the same was observed on the following 2-3 days.

During the investigation of the changes in acidity we found that the subcutaneous injection of pituitrin led to an insignificant decrease in the free hydrochloric acid content (by 8-12%) and an increase of roughly the same magnitude in the content of combined hydrochloric acid. We observed the same changes after the intravenous injection of pituitrin (irrespectively of the time of injection), but in a more pronounced form. The changes in the total hydrochloric acid content and the total acidity, irrespectively of the mode of injection, did not extend beyond the limits of variation in the control experiments. In view of these findings, it may be assumed that the content of combined hydrochloric acid increased at the expense of a decrease in the content of free hydrochloric acid. Irrespective of the mode of injection of pituitrin, no changes in the acidity could be detected on the day after injection of the preparation.

After the subcutaneous and, especially, the intravenous injection of pituitrin, the specific gravity of the gastric juice showed an increase. Whereas normally its value was 1.009-1.012, after injection of pituitrin it rose to 1.018-1.030. A very slight increase in the specific gravity was also observed on the next day.

Because of the increase in the specific gravity, we investigated the action of pituitrin on the content of dry residue. It was found that the content of dry residue increased irrespectively of the mode of injection of the pituitrin, but after intravenous injection the increase was more sharply expressed. The increase in the dry residue, like that in the specific gravity, was observed also on the following 2-4 days.

When the content of organic and inorganic substances in the dry residue was studied, it was found that the increase in the total dry residue took place mainly at the expense of organic substances, for the content of inorganic substances increased only very slightly.

It must be mentioned that pituitrin, when injected intravenously, caused sharper and more prolonged changes in the quantity and quality of the gastric juice than when injected in the same dose, but subcutaneously. This was evidently because absorption of pituitrin from the subcutaneous cellular tissue into the blood stream takes place gradually; as a result of this, its concentration in the blood does not reach high values, the more so because when the pituitrin does enter the blood it is partially destroyed. After intravenous injection, the concentration of pituitrin in the blood at once reaches a high level, which leads to the stronger effect of the action of the preparation.

Among the extensive literature reports there is no unanimity about the mechanism of action of pituitrin on the gastric glands. An attempt has been made [14] to explain the diminished secretion resulting frm the action of hypophysin by constriction of the blood vessels in the abdominal cavity and to a reduction in the blood supply of the stomach. In another paper [15], the same author considers that hypophysin acts on the secreto-depressor fibers of the splanchnic nerve.

Several authors [3,11,12,13] account for all the changes in the gastric secretion by an anemia of the stomach arising from the action of vasopressin. The opinion has also been expressed [7] that the action of hypophyseal preparations in depressing secretion "depends on the direct influence of the hypophyseal hormone on the gland cell or on its nerve endings".

It may be postulated that in the mechanism of action of pituitrin a part is played both by its influence on the vessels of the gastric mucosa, causing anemia, and by changes in the trophism of the glandular apparatus. Our hypothesis is supported by the fact that in all the experiments the changes in the qualitative composition of the juice were more prolonged than the quantitative changes.

These findings clearly show that pituitrin causes considerable and lasting changes in the working of the glandular apparatus of the stomach.

From the results obtained, the suggestion can be made to clinicians that patients suffereing from hypochlorhydric and achlorhydric gastritis, and also patients with dyspeptic disorders and other chronic diseases of the stomach, should be given pituitrin with great care, and in general its use in these patients should be avoided, for it should be replaced by drugs with a similar action, but which do not interfere with the working of the gastric glands.

SUMMARY

This work dealt with the study of the effect of pituitrin on the activity of gastric glands in Pavlov and Heidenhain stomach pouches. The volume of juice is decreased with administration of pituitrin. The latter also reduced the protease activity and increased the lipase activity of the gastric juice: The amount of free hydrochloric acid is diminished and that of the combined hydrochloric acid rises; however, the total hydrochloric acid content and the total acidity remain unchanged. Both specific gravity and dry residue are increased, the latter on account of organic substances.

LITERATURE CITED

[1] S. O. Badyl'kes, Transactions of the Seventh Congress of Russian Physicians (Moscow—Leningrad, 1925), p. 372 [In Russian].

[2] I. I. Burachevskii, Byull. Eksptl. Biol. i Med. 11, 2, 127 (1941).

[3] S. M. Dionesov, Fiziol. Zhur. SSSR 20, 3, 405 (1936).

[4] S. M Dionesov, The Role of Hormones in the Reaction of the Stomach to Painful Stimulation (Moscow, 1948) [In Russian].

[5] L. N. Karlik, the Role of the Hypophysis in Physiology and Pathology in the Light of Experiment (Moscow-Leningrad, 1939) [In Russian].

[6] M. Yu. Rapoport, Terap. Arkh. 10, 1, 65 (1932).

[7] D. M. Rossiiskii, Byull. Eksptl. Biol. i Med. 16, 3, 19 (1943).

[8] D. M. Rossiiskii, A. I. Kantorovich, I. Ya. Karmazin, et al., Problemy Endokrinol. i Gormonoterap. 4, 331 (1936).

[9] S. M. Ryss, Terap. Arkh. 10, 3, 294 (1932).
[10] G. M. Shershevskii, Klin. Med. 11, 3-4, 170 (1933).
[11] A. Bergman, cited by S.M. Dionesov.
[12] W. C. Cutting, E. C. Dodds, R. Noble et al., Proc. Roy. Soc. London, S. B. 123, 27 (1937).
[13] E. C. Dodds and R. C. Noble, Proc. Roy. Soc. Med. 30, 815 (1937).
[14] A. Elkeles, Ztschr. ges. exper. Med. 51, 147 (1926).
[15] A. Elkeles, Arch. Verdaungskr. 40, 380 (1927).
[16] H. Hoffmann, Ztschr. ges. exper. Med. 12, 134 (1921)
[17] B. A. Houssay, cited by S. M. Dionesov.
[18] J. Pal, Deutsche. med. Wchschr. 42, 1030 (1916).
[19] M. Testelin, cited by S. M. Dionesov.
[20] L. Zinino, cited by S. M. Dionesov.

THE RELATIONSHIP BETWEEN THE CONCENTRATIONS OF THYROTROPIC HORMONE IN THE HYPOPHYSIS AND THE PERIPHERAL BLOOD

R. D. Vyazovskaya and V.V. Mamina

Division of Histophysiology (Head—Honored Scientist Prof. B. V. Aleshin) of the Ukrainian Institute of Experimental Endocrinology (Director—Candidate Med. Sci. S. M. Maksimov), Khar'kov

(Presented by Active Member AMN SSSR V. V. Parin)

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No final solution has been reached of the problem of whether the anterior lobe of the hypophysis is a storage gland in which the processes of production and secretion of hormones may occur at different times, or whether the hormones produced enter the peripheral blood continuously.

The results of clinical observations and experimental investigations give the impression that the hypophysis is a gland of "continuous action" and that the hormones which it produces enter the blood stream immediately. If this hypothesis is correct, changes in the quantity of the various hormones in the hypophysis must be correspondingly reflected in their content in the blood. Experimental research has in fact shown that in rats after thyroidectomy the content of thyrotropic hormone increases in both the hypophysis and the blood serum [12]. In electrolytic lesions of the anterior hypothalamus, a decrease in the content of thyrotropic hormone in the anterior lobe of the hypophysis was accompanied by a corresponding fall in the level of this hormone in the blood [10].

After the prolonged administration of goitrogenic substances to animals, many authors found an increase in the concentration of thyrotropic hormone in the hypophysis [2,3,7]. Under these circumstances an increase in the concentration of the thyrotropic hormone in the blood is also observed [7-9].

Feeding rats on thiourea or sulfadianisidine [12] caused a decrease in the content of thyrotropic hormone in both the hypophysis and the blood serum. The different level of the thyrotropic hormone concentration in the hypophysis of females and males in summer and winter is reflected by corresponding changes in the concentration of this hormone in the blood [13].

In the present investigation our object was to determine whether changes in the content of thyrotropic hormone in the hypophysis are accompanied by parallel changes in its concentration in the blood.

METHOD

In view of data showing an increase in the content of thyrotropic hormone in the hypophysis 10 days after removal of the superior cervical sympathetic ganglia in rabbits [1,4,6] or after feeding these animals with 6-methylthiouracil [2,3], we first practised removal of the superior cervical sympathetic ganglia, and secondly, administration of 6-methylthiouracil (6MTU).

Our previous investigations on rabbits showed that a decrease in the content of thyrotropic hormone in the hypophysis may be caused by a combination of chronic stimulation of the sciatic nerve with feeding with 6MTU [5], and also by administration of chlorpromazine for 10 days (R. D. Vyazovskaya). We therefore fed the experimental animals with 6 MTU in association with stimulation of the sciatic nerve or administration of chlorpromazine.

Experiments were carried out on 24 male rabbits, weighting 2-2.5 kg. The concentration of thyrotropic hormone in the hypophysis and blood was estimated in guinea pigs. In all 30 guinea pigs were used.

The superior cervical sympathetic ganglia were removed in a one-stage surgical operation, without anesthesia. For 20 days the animals received 6MTU by mouth in a dose of 250 mg/kg body weight daily. Chronic stimulation of the sciatic nerve was applied through a silver wire in the upper third of the thigh, for 30 days. These rabbits received 6 MTU in the above dose for 20 days, starting on the 10th day after operation. Chlorpromazine was given by mouth in a dose of 20 mg/kg body weight for 10 days. At the end of the experimental period the rabbits were sacrificed by air embolism.

In order to determine the concentration of thyrotropic hormone in the hypophysis, guinea pigs of the 3rd and 4th series were grafted subcutaneously on each of 2 successive days with the hypophysis of rabbits which had

Concentration of Thyrotropic Hormone in the Hypophysis and Blood of Rabbits

Treatment given (donors)	Height of cells of thyroid epithelium of recipient guinea pigs in (μ) after receiving: extract of rabbits hypophyses			rabbit's blood		
	control	experiment	criterion of significance	control	experiment	criterion of significance
Removal of the superior cervical sympathetic ganglia		12.396±0.089 11.676±0.098 M=12.036±0.093	19.0		8.202±0.069 9.06±0.082 11.280±0.098 8.07±0.065 M=9.156±0.076	7.7
Administration of chlorpromazine	9.51±0.096 9.58±0.09 M=9.545±0.093	7.548±0.079 8.97±0.085 M=8.259±0.082	10.3	8.604±0.075 7.992±0.077 8.671±0.083 8.256±0.079 M=8.31±0.076	7.686±0.058 7.44±0.084 8.976±0.090 6.996±0.053 M=7.77±0.071	5.2
Administration of 6MTU		10.87±0.10 10.65±0.10 M=10.76±0.10	24.7	7.18±0.057 7.22±0.048 6.88±0.055 8.45±0.073	9.20±0.088 8.09±0.096 9.37±0.099 8.24±0.077 M=8.7±0.090	11.8
Stimulation of the sciatic nerve + 6MTU	7.8±0.065 7.83±0.066 M=7.81±0.065	5.65±0.065 6.21±0.066 M=5.93±0.065	20.6	M=7.43±0.058	5.48±0.053 5.76±0.055 4.70±0.057 5.41±0.057 M=5.33±0.055	26.2

received the same treatment. The recipients were sacrificed 100 hours after the first graft. The 1st and 2nd series of guinea pigs received injections of rabbit hypophysis, preliminarily dried in acetone, in the form of a suspension in physiological saline, for 4 days in a total dose of 2 hypophyses per guinea pig. The animals were sacrificed 72 hours after the first injection. When the latter method was used, a much more intensive reaction was observed in the thyroid gland of the guinea pigs than after grafting of the hypophysis.

Thyrotropic hormone was precipitated from the blood of the rabbit by Fellinger's method [11], and assayed simultaneously in two guinea pigs. The criterion of the content of thyrotropic hormone in the hypophysis or blood was the change in the mean value of the height of the epithelial cells of the thyroid gland of the guinea pig (recipient) receiving the hypophysis or blood extract.

Because of seasonal variations in the concentration of thyrotropic hormone in the hypophysis, in each series we determined the concentration of this hormone in control rabbits concurrently with the determinations in the experimental animals. Series which were done at the same time had a common control.

The mean figures obtained in the experimental groups were compared with the results established for the control rabbits of the particular series. The values obtained were treated by the methods of variational statistics. The difference between the experimental and control values was regarded as significant if the value of the criterion of significance r was equal to or greater than 3 ($r \geq 3$)*.

RESULTS

In the first place we studied the height of the cells of the thyroid epithelium in intact guinea pigs sacrificed at different periods of the year; in the autumn-winter period its value was 5-5.5μ and in the spring-summer period it was 4-4.8μ.

The figures in the table show that removal of the superior cervical sympathetic ganglia in rabbits led to an increase in the concentration of thyrotropic hormone in the hypophysis by comparison with the controls. Whereas the mean height of the cells of the epithelium of the thyroid gland of the recipient guinea pigs M after receiving injections of a suspension of the hypophyses of intact control rabbits was 9.545μ, after receiving injections of hypophyses of rabbits undergoing ganglionectomy this value was 12.036μ. This increase was significant, for the criterion of significance r = 19.

The level of thyrotropic hormone in the blood of the rabbits was correspondingly higher (M = 9.156μ) after removal of the superior cervical sympathetic ganglia than in the control animals, in which M = 8.381 (r = 7.7).

Administration of 6MTU led to an increase in the concentration of thyrotropic hormone in the hypophysis of the experimental rabbits by comparison with the controls, for the mean height of the cells of the thyroid epithelium of the recipients M after grafting of the hypophyses of rabbits receiving 6MTU was 10.76μ; the hypophyses of the control (intact) rabbits in this series caused an increase in the height of the cells of the thyroid epithelium of the recipients to an average of 7.81μ. This difference was statistically significant, for r = 24.7.

Whereas after the action of 6MTU the concentration of thyrotropic hormone in the hypophysis was raised, the concentration of this hormone in the blood of these donor rabbits was higher (M = 8.7μ) than in the control series tested at the same time, in which the height of the cells of the thyroid epithelium of the recipients was on the average 7.77μ (r = 11.8).

After administration of 6MTU in association with stimulation of the sciatic nerve, the concentration of thyrotropic hormone in the hypophysis of the rabbits was lowered (M = 5.93μ) by comparison with the controls, in which M = 7.81μ (r = 20.6). Corresponding to the lowered concentration of thyrotropic hormone in the hypophysis, the concentration of this hormone in the blood was also lowered in these animals (M = 5.33μ) by comparison with the level in the control animals (M = 7.43μ). The criterion of significance of the lowering of the concentration of this hormone in the blood r = 26.2.

Injection of a suspension of hypophyses of rabbits receiving chlorpromazine into recipient guinea pigs caused a much smaller increase in the height of the cells of the thyroid epithelium (M = 8.259μ) than in the case of control animals, in which M = 9.545μ (r = 32.8). When the blood of rabbits receiving chlorpromazine was tested it was also found that the concentration of thyrotropic hormone in it was lower than that in the blood of the control animals. In the first case the height of the cells of the thyroid epithelium of the recipients M = 7.77μ, and in the second case M = 8.381μ. The fall was significant, since r = 5.2.

It follows from analysis of the results obtained that a change in the concentration of thyrotropic hormone in the hypophysis is reflected by a corresponding change in its concentration in the blood. An increase in the concentration of the hormone in the hypophysis after removal of the superior cervical sympathetic ganglia, or administration of 6MTU, for instance, was accompained by an increase in the concentration of this hormone in the peripheral blood, whereas a lowered concentration of thyrotropic hormone in the hypophysis (resulting from the action of 6MTU in association with stimulation of the sciatic nerve or of the action of chlorpromazine) corresponded to small concentrations of this hormone in the blood also. Hence it follows that the thyrotropic hormone, after its synthesis in the hypophysis, is not retained in the parenchyma of the gland but at once enters the blood stream.

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* The criteria of significance r were determined from the formula: $$r = \frac{M_{expt} + M_{control}}{\sqrt{m^2_{expt} + m^2_{control}}}$$ where M is the mean height of the cells of the thyroid epithelium of the guinea pigs; m^2 is the mean square error.

The anterior lobe of the hypophysis must therefore be regarded as a gland of merocrine type, with continuous secretion.

SUMMARY

Simultaneous determinations of the content of thyrotropic hormone demonstrated a clear-cut parallelism between the shifts in the concentration of this hormone appearing in the hypophysis and the peripheral blood of rabbits.

The rise of the thyrotropic hormone content in the hypophysis, observed after removal of the upper cervical sympathetic ganglia or after administration of 6-methylthiouracil, was accompanied by an increase of the level of this hormone in the peripheral blood.

On the other hand reduction in the concentration of the thyrotropic hormone in the hypophysis during the administration of 6-methylthiouracil while, at the same time, stimulating the sciatic nerve, or after administration of chlorpromazine corresponded to a decrease in the level of the hormone in the peripheral blood.

LITERATURE CITED

[1] B. V. Aleshin and R. D.Vyazovskaya, Transactions of a Conference on the Role of Neurohumoral and Endocrine Factors in the Activity of the Nervous System in Normal and Pathological Conditions (Moscow—Leningrad, 1959) p. 122 [In Russian].

[2] B. V. Aleshin and N. S. Demidenko, Arkh. Anat., Gistol., i Embriol. 29, 3, 82 (1952).

[3] B. V. Aleshin and N. S. Demidenko, Arkh. Anat., Gistol., i Embriol. 30, 5, 31 (1953).

[4] B. V. Aleshin and S. P. Nilolaichuk, Transactions of the Fifth All-Union Congress of Anatomists, Histologists, and Embryologists (Leningrad, 1951) p. 655, [In Russian].

[5] V. V. Mamina, Proceedings of a Scientific Session of the Ukrainian Institute of Experimental Endo crinology and the Kharkov Society of Endorcrinologists on the Physiology and Pathology of the Thyroid and Sex Glands (Khar'kov, 1955) p. 68 [In Russian].

[6] S. P. Nikolaichuk, Prodeedings of a Jubilee Scientific Session to Celebrate the 25th Anniversary of the Founding of the Ukrainian Institute of Experimental Endocrinology (Khar'kov, 1945) p. 44 [In Russian].

[7] S. A. D'Angelo, Endocrinol. 52, 331 (1953).

[8] S. A. D'Angelo, Endocrinol. 56, 37 (1955).

[9] S. A. D'Angelo, C. E. Stevens, K. E. Paschkis et al., Endocrinol. 54, 565 (1954).

[10] S. A. D'Angelo, R. E. Traum. Endocrinol. 59, 593 (1956).

[11] K. Fellinger, Wien Arch. inn. Med. 29, 375 (1936).

[12] A. S. Gordon, E. D. Goldsmith and H. A. Charipper, Endocrinol. 36, 53 (1945).

[13] F. A. Soliman and Y. S. Ghanem, Nature 179, 102 (1957).

THE EFFECT OF DENERVATION OF THE SPLEEN ON THE SERUM VITAMIN B_{12} LEVEL IN DOGS

V. F. Markelova

Laboratory of General Physiology of the Institute of Normal and Pathological Physiology (Director—Active Member AMN SSSR V. N. Chernigovskii), AMN SSSR, Moscow

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

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The influence of the nervous system of the circulation of the blood has been widely studied in recent years by Soviet researchers [7]. Numerous investigations [3-7] have shown that after denervation of certain internal organs, changes develop in the peripheral blood picture and in the bone marrow.

A prominent place among these investigations is occupied by studies of the effect of denervation of the spleen in the activity of the blood system. It has been shown [1,3-7] that, after denervation of the spleen, anemia develops in every case. The times of development and the character of the anemias observed by different authors, however, have not shown any uniformity.

I. S. Rozanova and E. A. Zhukova [6], for instance, observed a decrease in the hemoglobin concentration and the red cell count only during the first two weeks after denervation of the spleen. These indices subsequently rapidly regained their original level, whereas the changes in the bone marrow continued to progress.

E. L. Kan [5] observed a hypochromic anemia in cats after denervation of the spleen, with the maximum fall in the hemoglobin concentration and red cell count on the 8th-14th day after operation, and lasting for several months.

R. A. Durinyan [3] showed that in cats this operation leads to the development of a hypochromic microcytic anemia of regenerative type. This author also pointed out the existence of a wide amplitude of variations in the red cell count and the hemoglobin level throughout the duration of the anemia.

According to N. N. Beller's findings [1], after denervation of the spleen in the cats the anemia follows a characteristically undulating course, and develops as a hyperchromic type.

Finally, M. A. Kalnyn' [4] observed in dogs an anemia with a maximum fall in the hemoglobin level and red cell count on the 9th-14th day after denervation of the spleen, and lasting for 2-3 months.

Denervation of the spleen thus leads to the development of anemia. In some cases the fall in the hemoglobin level and the red cell count is slight and the anemia does not last longer than 2-3 weeks, but in others, on the contrary, a considerable and prolonged fall is observed in these indices; some authors have observed anemia of a hypochromic character, others hyperchromic.

In the present investigation we studied the influence of denervation of the spleen on the vitamin B_{12} concentration in the serum of dogs, i.e. the changes in the blood concentration of a powerful antianemia factor such as vitamin B_{12} during the development of experimental anemia.

METHOD

In chronic experiments on dogs (males), we studied the hemoglobin concentration (Sahli), the red and white cell counts, and the vitamin B_{12} concentration before and after operation. Blood was taken daily, from the veins of the hind limb, with the animal in a fasting condition.

When we calculated the color index for each animal we used the mean values of the hemoglobin concentration and the red cell count obtained from examination of the blood before operation.

The determination of vitamin B_{12} was based on a microbiological method [2] in which a strain of Escherichia coli, obtained from Prof. V. I. Bukin's laboratory, was utilized as test orgainsm. After seeding on a liquid medium containing vitamin B_{12}, the strain of E. coli gave increased turbidity, which was proportional to the concentration of vitamin B_{12} in the medium. Estimation of the turbidity was carried out by means of a photoelectric colorimeter, using a light blue filter.

After the normal fluctuations in the above indices had been studied for 4 to 6 weeks, in 5 dogs the spleen was denervated. The operation was performed under general ether anesthesia. All the nerve branches in the main vascular bundle of the spleen were divided. The vessels were exposed, the adventitia was carefully removed and their walls were then painted with 10% phenol solution.

As a control operation, laparotomy was performed, followed by dissection of the corresponding nerves and

Table 1. Changes in the Blood Composition and the vitamin B_{12} Concentration in a in a Control Dog, not Undergoing Operation

Serial no.	Date of observation	Hemoglobin concentration (Sahli) in %	Red cell count (in thousands)	Color index	White cell count	Vitamin B_{12} concentration, mμg/ml
	1956					
1	7/XII	78	6220	1.04	9000	0.400
2	11/XII	78	6780	0.93	9500	0.432
3	18/XII	76	6055	1.046	10000	0.372
4	25/XII	82	6900	0.99	8700	0.334
	Mean	78	6490	—	9300	0.384
	1957					
6	7/I	80	6900	0.96	8000	0.390
7	15/I	80	6870	0.96	9450	0.468
8	24/I	81	7000	0.96	8700	0.456
9	5/II	82	7200	0.94	7900	0.372
10	9/II	80	7000	0.95	9600	0.252
11	20/II	82	7125	0.95	1000	0.314
12	25/II	83	7200	0.96	9500	0.334
13	5/III	83	6850	1.00	9000	0.390
14	13/III	82	6900	0.99	8250	0.372
15	20/III	84	7000	1.00	9300	0.390

vessels of the spleen, which were not, however, divided.

In one dog, in addition, without any operative procedure of any kind, the blood indices were systematically studied in order to show any possible changes in the blood picture associated with the conditions under which the animals were kept, or any seasonal variations.

The investigations of the experimental and control animals lasted 5-6 months.

RESULTS

Variations in the blood indices of the dogs before operation were slight. The maximum amplitude of the variation in the red cell count, hemoglobin level, white cell count and vitamin B_{12} concentration was 800,000, 2-5%, 1000 and 0.1-0.2 mμ g/ml respectively.

No essential changes were observed in the blood picture of the control animal, on which no operation was performed, throughout the period of investigation (Table 1). The control operation led to a brief (7-8 days) and slight fall in the red cell count and hemoglobin level, but had no effect on the serum vitamin B_{12} concentration (Table 2).

The red cell count thus fell by 1,200,000, the hemoglobin concentration by 12%, and the white cell count on the average was doubled.

Denervation of the spleen caused a more prolonged and pronounced change in the blood picture. In all the experimental dogs, during the first two weeks after the operation, a fall in the red cell count by on the average 1,575,000, and in the hemoglobin concentration by 16% took place; the white cell count was increased two- or threefold. On the 15th-20th day all these indices returned to their initial values or even slightly exceeded them, and remained at a higher level than before the operation throughout the period of investigation.

On careful examination of the results obtained (Table 3), it will be seen that the most characteristic change in the blood picture of the animals undergoing denervation of the spleen was the presence of considerable fluctuations in the values of the indices investigated from one experiment to another. It must be mentioned that the amplitude of the variations in the red cell count was greater than the amplitude of variation of the hemoglobin concentration, which resulted in periodic changes in the color index. As shown by the figures in Table 3, the variation in the vitamin B_{12} concentration in the serum before operation could be measured in tenth parts of a mμ g/ml, whereas after operation the vitamin B_{12} concentration changed considerably (three- or fourfold) from one determination to another.

We were unable to show any parallel trend in the increases in the red cell count and vitamin B_{12} concentration, and in many determinations, when an increase or decrease was observed in the vitamin B_{12} concentration, the red cell count was decreased or increased respectively.

It is apparent from the figures given that after denervation of the spleen in dogs the development of a true anemia is not in fact observed, but there is merely a

TABLE 2. Changes in the Composition of the Blood and the Vitamin B_{12} Concentration in a Control Dog Undergoing Laparotomy

Serial no.	Date of observation	Hemoglobin concentration (Sahli) in %	Red cell count (in thousands)	Color index	White cell count	Vitamin B_{12} concentration mμg/ml
	1956					
1	18/XII	75	6 640	0.99	8 250	0.504
2	20/XII	80	6 600	1.07	9 300	0.480
3	25/XII	76	6 800	0.98	8 000	0.390
	1957					
4	2/I	76	7 000	0.958	9 000	0.468
	Mean	76.9	6 760		8 638	0.460
	1957					
6	3/I		Laparotomy performed			
7	7/I	65	5 300	1.08	14 000	0.444
8	15/I	70	6 500	0.95	9 000	0.456
9	24/I	76	6 900	0.96	7 800	0.390
10	29/I	76	6 500	1.03	8 500	0.366
11	3/II	80	7 000	1.00	9 300	0.468
12	9/II	75	6 600	0.99	9 600	0.366
13	20/II	76	6 800	0.48	8 750	0.408
14	28/II	76	7 000	0.95	8 900	0.390
15	5/III	78	6 400	1.07	9 300	0.375
16	13/III	80	6 800	1.00	10 000	0.314
17	20/III	79	6 600	1.05	9 700	0.390
18	27/III	76	7 000	0.95	9 300	0.400
19	2/IV	78	6 500	1.06	8 500	0.390
20	9/IV	75	6 600	0.99	9 500	0.420
21	24/IV	77	6 900	0.98	9 250	0.318
22	7/V	76	6 950	0.97	8 750	0.368

slight and brief fall in the hemoglobin level and the red cell count.

It must be mentioned that our findings differ considerably from those in the literature [1,3-5]. This discrepancy may probably be accounted for by the fact that our investigations were carried out on dogs, in contrast to those of the previous authors, who performed their experiments on rabbits and cats. It is also known that the results obtained in experiments on the same species of animal [1,3,4,6,] differ considerably from each other. For example, the degree, character, duration, and course of the anemias observed by different authors as a result of denervation of the spleen in cats are not the same. It may be suggested that denervation of the spleen in dogs does not lead to such profound changes in hemopoiesis as are observed in cats.

It is difficult for us to give a convincing explanation of the discrepancy between our own results and those of M. A.Kalnyn' [4], who, like us, carried out experiments on dogs and observed the development of anemia in these animals after denervation of the spleen.

On the question of the interpretation of experimental anemias, N. N. Beller [1] points out that the term "anemia" applies also to observations in which the red cell count and the hemoglobin level fell relatively to their initial values, but in absolute values these indices were maintained only a little below their mean values. If this definition is used as a starting point, in our own cases we may speak of the appearance of a brief anemia which, in the 3rd week, gave way to a slight increase in the level of these indices by comparison with the original values.

In our opinion the most characteristic feature of the blood picture in dogs after denervation of the spleen is the presence of fluctuations in the hemoglobin concentration and, in particular, of the red cell count and the vitamin B_{12} concentration from one determination to another. This feature suggests that denervation of the spleen leads to a disturbance of the mechanism maintaining these indices in normal conditions at a more or less stable level.

SUMMARY

An insignificant anemia of short duration develops following splenic denervation in dogs. The most characteristic feature of the blood picture in this condition is

TABLE 3. Changes in the Composition of the Blood and the Vitamin B_{12} Concentration after Denervation of the Spleen in the Dog

Serial no.	Data of observation	Hemoglobin concentration (Sahli) in %	Red cell count (in thousands)	Color index	White cell count	Vitamin B_{12} concentration mμg/ml
	1956					
1	18/XII	78	7 100	0.94	8 500	0.318
2	20/XII	78	6 640	1.01	9 000	0.420
3	25/XII	79	6 900	0.98	9 700	0.368
	1957					
4	2/I	80	6 600	1.04	9 500	0.385
	Mean	78.75	6 810		9 175	0.373
	1957					
6	3/I	Operation of denervation of the spleen				
7	7/I	60	5 050	1.03	17 600	1.4
8	15/I	65	6 000	0.92	12 500	0.264
9	24/I	68	6 900	0.84	13 600	0.111
10	29/I	76	7 100	0.92	10 500	318
11	5/II	80	6 000	1.14	9 200	0.129
12	9/II	80	6 800	1.01	10 300	0.202
13	20/II	86	7 400	1.00	9 000	0.420
14	28/II	83	6 400	1.01	9 625	0.108
15	5/III	86	7 000	1.05	8 700	0.420
16	13/III	80	6 200	1.10	7 000	0.111
17	20/III	80	7 000	0.9	7 000	0.129
18	27/III	79	6 200	1.00	9 000	0.420
19	2/IV	77	7 100	0.90	9 500	0.385
20	9/IV	73	6 200	1.00	10 000	0.204
21	17/IV	83	7 400	0.9	9 000	0.385
22	24/IV	85	7 500	0.9	10 500	0.186
23	7/V	85	7 500	0.9	8 250	0.132
24	14/V	73	6 250	1.00	11 000	0.480

the presence of fluctuations in the hemoglobin level and especially in that of the red cell count and vitamin B_{12} concentration, varying from one test to another. It is suggested that splenic denervation tends to disturb the mechanism maintaining a more or less stable level of the mentioned indices within the normal limits.

LITERAUTRE CITED

[1] N. N. Beller, The Participation of the Nervous System in the Regulation of the Composition of the Blood and the Oxygen Saturation of the Arterial Blood during Anoxia. Candidate's dissertation (Leningrad, 1957) [In Russina].

[2] V. N. Bukin, L. Ya. Areshkina and L. S. Kutseva, Biokhimiya 19, 713 (1954).

[3] R. A. Durinyan, Experimental Analysis of Anemias Caused by the Denervation of Certain Internal Organs. Candidate's dissertation (Moscow, 1956) [In Russian].

[4] M. A. Kalnyn', The Effect of Denervation of the Spleen on the Processes of Hemopoiesis. Author's summary of candidate's dissertation (Riga, 1955) [In Russian].

[5] E. L. Kan, Data on the Study of the Influence of the Nervous System on the Composition of the Blood. Candidate's dissertation (Leningrad, 1953) [In Russian].

[6] N. S. Rozanova and E. A. Zhukova, Present Problems of Hematology and Blood Transfusion (Moscow, 1955) 31, p.85 [In Russian].

[7] V. N. Chernigovskii and A.Ya. Yaroshevskii, The Nervous Regulation of the Blood System (Moscow, 1953) [In Russian].

CHANGES IN HEMOPOIESIS IN CONGESTIVE SPLENOMEGALY WITH OR WITHOUT DENERVATION OF THE SPLEEN

I. Ya. Ashkinazi

Clinic of Propedeutics of Internal Diseases (Head—Active Member AMN SSSR M. D. Tushinskii) and the Department of Pathological Physiology (Head—Prof. M. M. Pavlova), I. P. Pavlov First Leningrad Medical Institute

(Presneted by Active Member AMN SSSR M. D.Tushinskii)

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The problem of the mechanism of the changes in the composition of the blood in clinical and experimental congestive splenomegaly still continues to be a topic of discussion. Certain authors have suggested a possible reflex influence of the pathologically changed spleen on the hemopoietic system [4].

Of great interest are the reports of histologists [1] of the development of degenerative changes in the nerve cells of the solar plexus in splenomagaly, which in itself may be of importance in the development of the disorders of hemopoiesis. Experimental researches of Soviet authors [2,3] have given evidence of the possible development of anemia after denervation of the spleen.

We have been unable to find in the literature any mention of the study of the role of the nervous system in the production of the disorders of hemopoiesis in the splenomegalies.

The aim of the present research was to study the effect of ligation of the splenic vein (congestive splenomegaly) on hemopoiesis in digs and to ascertain the importance of denervation of the spleen in the mechanism of development of these changes.

METHOD

The investigations were carried out on 5 male dogs over a period of 7-11 months.

Ligation of the splenic vein was performed in two dogs, and in two more, ligation of the vein was combined with denervation of the spleen; in one dog denervation of the spleen alone was performed.

Congestive splenomegaly was produced by ligation of the main venous trunk and all the small veins composing the hilum. In denervation of the spleen, all the visible nerves leading to the spleen were divided. The adventitia was removed from the walls of the arteries for a distance of 2 cm, and the walls were then painted with a 10% solution of carbolic acid. In the course of the experiment we determined the red cell count, the hemoglobin concentration, the white cell count and formula, the platelet count, the osmotic resistance of the red cells to a hypotonic solution of NaCl and the serum bilirubin (Jendrassik's method), and we examined the bone marrow from puncture specimens.

In the present communication we give the results of observations over a period of 6 months.

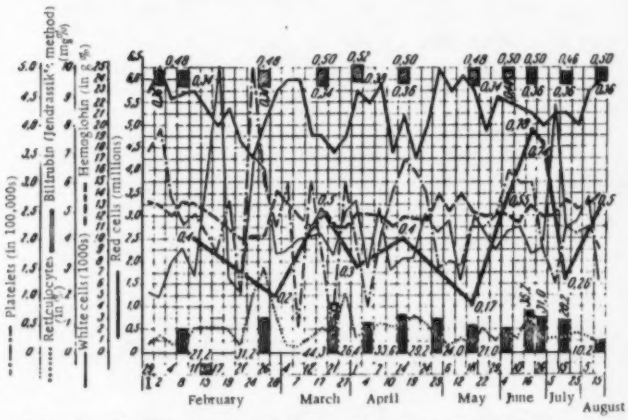

Fig. 1. Changes in the indices of hemopoiesis after ligation of the splenic vein (the dog Zhuk). February 15, 1958 — ligation of the splenic vein. Legend: ■ — number of erythroblasts in the bone marrow, in %; ▨ — osmotic resistance of the red cells to hypotonic saline.

RESULTS

In Fig. 1 are shown details of the changes in the peripheral blood picture and in the bone marrow after ligation of the splenic vein in the dog Zhuk. Immediately after operation a normochromic anemia developed, with a chronic remittent subsequent course. The periods of anemia were accompanied by an increase in the reticulocyte count, increased growth of cells of the erythroid series in the bone marrow, and a relative increase in the serum bilirubin concentration. In individual investigations a fall occurred in the osmotic resistance of the red cell. The development anemia was accompanied by a leukocytosis with an increase in the

number of segmented neutrophils, a slight eosinophilia during the first month, and a transient rise in number of monocytes and cells of the reticulo-endothelial system, and also by variations in the platelet count, which showed a tendency to fall.

In the next dog (Okhotnik), the hemoglobin concentration before operation was 90-92%, the red cell count 5,6000,000-6,200,000, the color index 0.73-0.86, the reticulocytes 0.1-0.5%, the osmotic resistance of the red cells 0.46-0.34, the serum bilirubin 0.5mg%, the white cell count 7400-11,500, the platelet count 120,000-230,000, and of the bone marrow cells, 11.8% were erythroblasts.

Immediately after ligation of the splenic vein a progressive anemia began to develop and on the 6th day the red cell count was 3,000,000. In the course of 2 months a remittent anemia was maintained, with a gradual restoration of the normal red cell count at the beginning of the 3rd month.

The development of anemia was accompanied by reticulocytosis (6.8%) and by an increase to 50.2% in the number of erythroblasts in the bone marrow.

Starting with the 3rd month, in spite of the restoration of the normal red cell count, intermittent increases took place in the reticulocyte count of the peripheral blood to 2.1%, accompained by similar intermittent fluctuations in the number of erythroblasts in the bone marrow. In respect to leukopoiesis, an intermittent leukocytosis was observed, with an increase in the segmented neutrophils; lymphocytosis with a slight eosinophilia, and an increase in the monocytes and cells of the reticuloendothelial system in the course of the first month after operation. Throughout the whole period of the observations there were intermittent increases in the platelet count to 310,000-470,000.

When ligation of the splenic vein was combined with denervation of the spleed (the dog Sever), as can be seen from Fig. 2, for 2 months there was a normochromic anemia with a slight reticulocytosis and an increase in the number of erythroblasts in the bone marrow. Starting with the 3rd month, a progressive fall took place in the red cell count, with a tendency towards hyperchromia (color index 1.1-1.2), with a marked reticulocytosis (75%), sharp stimulation of the erythroid series in the bone marrow (80% of erythroblasts), and an increase in the serum bilirubin concentration to 0.95 mg%.

In view of the unremitting increase in the anemia, splenectomy was performed (August 5, 1958, see Fig.2), after which a rapid rise took place immediately in the red cell count, with a simultaneous decrease to normal figures in the reticulocyte count and the serum bilirubin concentration. Signs of stimulation of the erythroid series in the bone marrow disappeared.

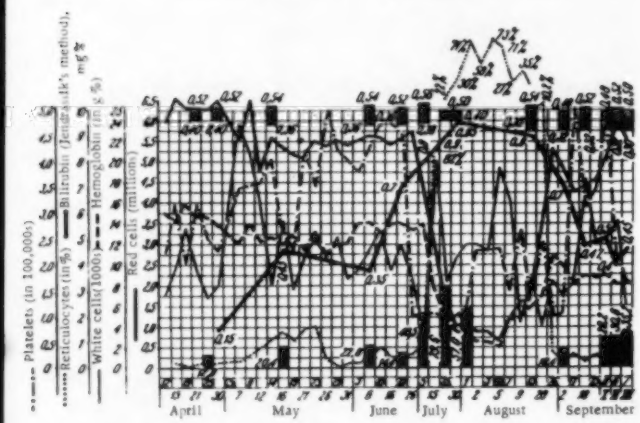

Fig. 2. Changes in the indices of hemopoiesis after ligation of the splenic vein, combined with denervation of the spleen (the dog Sever). May 15, 1958 — ligation of the splenic vein combined with denervation. August 5, 1958 — splenectomy. Legend as in Fig. 1.

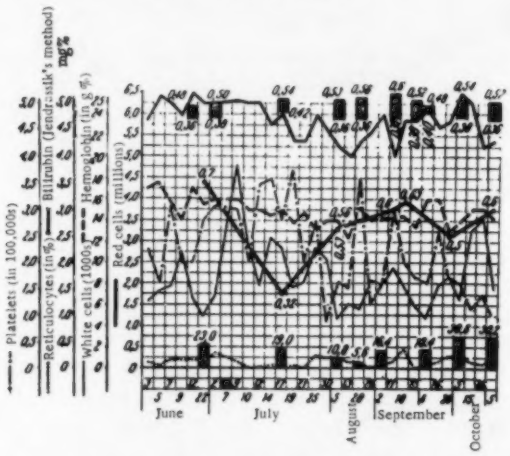

Fig. 3. Changes in the indices of hemopoiesis after denervation of the spleen (the dog Ryzhii). July 7, 1958 — denervation of the spleen. Legend as in Fig. 1.

Subsequently, as is apparent from Fig. 2, besides a slight fall in the red cell count, a considerable lowering of the hemoglobin concentration was observed (color index 0.5-0.4). In the bone marrow at this stage there was an increase in the number of erythroblasts with a relative increase in the proportion of basophilic forms.

These changes provide evidence in favor of inhibition of the maturation of erythroblasts and a diminution of the hemoglobinization of the red cells, evidently on account of a disturbance of the iron metabolism. Several authors have pointed out the possible development of such changes affecting hemopoiesis after splenectomy. At the height of development of the anemia, leukocytosis was observed with eosinopenia, lymphocytopenia and a considerable increase in the number of monocytes and cells of the reticulo-endothelial system. Thrombocytopenia was also observed. After splenectomy the normal indices of leuko- and thrombopoiesis were restored.

In another dog (Seryi), on which a similar operation was performed, the preoperative findings were: hemoglobin 74.4-90%, red cells 5,700,000-6,300,000, reticu-

locytes 0.1-0.8%, osmotic resistance of the red cells 0.50-0.36, bilirubin 0.7 mg%, white cells 7860-12,000; the bone marrow contained 21.4% of erythroblasts. After operation the development of a normochromic anemia was observed, with maximum decrease in the red cell count on the 13th day to 4,000,000. A moderate degree of anemia was maintained during the next two months. Starting with the middle of the second month, simultaneously with an increase in the red cell count, there was a reticulocytosis (1.9%) and stimulation of erythroblastic maturation in the bone marrow. This was not observed in the period of development of the anemia. After restoration of the normal red cell and reticulocyte counts, the number of erythroblasts in the bone marrow fell to normal figures.

A slight fall and rist in the maximum and minimum red cell resistance was observed. The bilirubin concentration was unchanged. For 2 months an increased white cell count to 46,000 was observed, which in the next months was around 14,000. The platelet count remained unchanged.

In the dog in which denervation of the spleen alone was performed (Ryzhii), from the 10th day after operation the animal developed a moderate normochromic anemia with a tendency towards intermittent fluctuations in the red cell count (Fig. 3). The development of anemia was accompanied by reticulocytopenia and by a fall in the number of erythroblasts in the bone marrow. A fall in the osmotic resistance of the red cells occurred in individual experiments. The bilirubin concentration was unchanged. For a period of 1/2-1 month the platelet count showed a tendency to rise and fall, and starting with the 3rd month a tendency towards thrombocytopenia was apparent.

The changes in the hematological indices in the experiments in which the splenic vein was ligated testify to the hemolytic character of the developing anemia (the rise in the reticulocyte count, the increased bilirubin concentration and the stimulation of erythropoiesis in the bone marrow in the absence of signs of disturbance of maturation of erythroblasts). In the experiments in which ligation of the vein was combined with denervationof the spleen, one dog (Sever) also developed a hemolytic anemia, but it was much less pronounced than in the dogs in which the vein was ligated only. In the other dog (Seryi) the development of anemia was not associated with intensification of hemolysis. It should be mentioned, however, that in this dog a small vein was left unligated.

During denervation of the spleen only, the development of anemia was due to depression of erythropoiesis (reticulocytopenia, decreased number of erythroblasts in the bone marrow), which agrees with the findings of other authors, working with dogs.

We consider that in congestive splenomegaly, when increased hemolysis of red cells takes place, denervation of the spleen may evidently increase this process still further.

It also follows from our observations that the presence of splenomegaly in its turn alters the character of the hematological changes usually observed in dogs after denervation of the spleen only.

SUMMARY

Following ligation of the splenic vein in two dogs, they showed development of wave-like hemolytic anemia. In one of the two dogs in which the vein was ligated in combination with splenic denervation, the hemolytic anemia manifested itself to a considerably greater degree than was the case in the experiment with the vein ligation alone. A rapid rise in the red cell count occured after splenectomy had been performed in connection with the progressive anemia. In dogs with splenic denervation alone, the development of anemia is connected with the depression of erythropoesis.

LITERATURE CITED

[1] D. Yu. Guseinov, The Pathomorphology of the Peripheral Nervous System of Receptors and Synapses (Baku, 1957) [In Russian].

[2] R. A. Durinyan, Experimental Analysis of the Anemias Caused by Denervation of Certain Internal Organs. Candidate's dissertation (Moscow, 1956) [In Russian].

[3] E. L. Kan, Byull. Eksptl. Biol. i Med. 37, 3, 29 (1954).

[4] E. I. Novoselova, The Pathogenesis of Leukopenia in Malaria (The Role of the Spleen in the Neurohumoral regulation of Hemopoiesis). Author's summary of dissertation (Moscow, 1956) [In Russian].

CHARACTERISTICS OF THE ANEMIA FOLLOWING DIVISION OF THE VAGUS NERVES

R. A. Durinyan

Laboratory of General Physiology (Head—Active Member AMN SSSR V. N. Chernigovskii) of the Institute of Normal and Pathological Physiology (Director—Active Member AMN SSSR V. N. Chernigovskii), AMN SSSR, Moscow

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

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The problem of the nervous regulation of the blood system has recently attracted the attention of many researcher. Much factual material has been collected and analyzed [1-4, 8, 9].

V. N. Chernigovskii and his co-workers have studied and described experimental anemias caused by denervation of certain internal organs [4,8,9]. In these investigations an important place is occupied by the study of the anemias arising as the result of partial denervation of the stomach by the formation of a gastric pouch by the Klemenciewicz-Heidenhain method [2,4,9].

In the present article we describe an experimental anemia caused by the bilateral subphrenic division of the vagus nerves.

METHOD

Experiments of long duration were performed on 3 experimental and 2 control dogs. The blood indices examined in all the dogs before and after operation included cell count, hemoglobin concentration, osmotic resistance, diameter and volume of the red cells (hematocrit), and examination of bone marrow films. We also studied the uptake of radioactive iron Fe^{59} by the red cells and made a morphological analysis of the gastric mucosa. The operation was performed under general ether anesthesia. Both vagus nerves were divided below the diaphragm, an annular strip of the mucous membrane was removed from the cardial portion of the stomach, and this area was then painted with a 10% solution of phenol. The control operation consisted of dissection of the vagus nerves in the cardial portion of the stomach. The surrounding tissues were painted with the same phenol solution. Before operation, and during the first 3 months after the operation, blood examinations were made on alternate days, and subsequently once every 3-4 days. The investigations lasted 10 months.

RESULTS

In Fig. 1 are shown the changes in the peripheral blood of the control dog Belyak. We can see that during the 5 months before operation the variations in the cell counts and the hemoglobin concentration were insignificant.. The osmotic resistance, diameter and volume of the red cells, and also the bone marrow picture, remained stable.

Radioactive iron Fe^{59} was found in the plasma 15-30 minutes after its administration, reached its maximum level after 120-240 minutes, and then began to fall off in concentration. Radioactivity was detected in the red cells on the 4th-5th day, reaching its maximum on the 8th-9th day, at which level it stayed for a long time, and then gradually decreased in intensity. After 4 months the Fe^{59} content of the red cells did not exceed 1%*.

After the control operation, the dog Belyak developed a transient hypochromic anemia. On the 5th day the red cell count fell by 900,000 and the hemoglobin level by 12%; the reticulocyte count increased fourfold. The remaining blood indices were unchanged. On the 10th day the number of red cells and reticulocytes and the hemoglobin percentage returned to their initial values (see Fig. 1). After the repeated administration of radioactive iron we obtained the same results. The changes which have been described were also obtained in the other control dog, Malysh.

More siginificant and lasting changes were observed in the blood picture and the bone marrow after division of the vagus nerves. In the dog Bars (Fig. 2), for instance, on the 10th day after division of the vagus nerves the red cell count fell by 2,000,000; the hemoglobin concentration at first rose, but by the 17th day it had fallen by 16% of its initial value; the white cell count rose sharply in the first few days, but gradually returned to its initial level; in the same period there was a threefold increase in the reticulocyte count, but at the end of the first month it returned to its initial value. At the beginning of the second month the red cell count rose, and was now 1,000,000-1,200,000 below its normal value.

*Byull. Eksptl. Biol. i Med. 3, 51-55 (1958); see CB translation

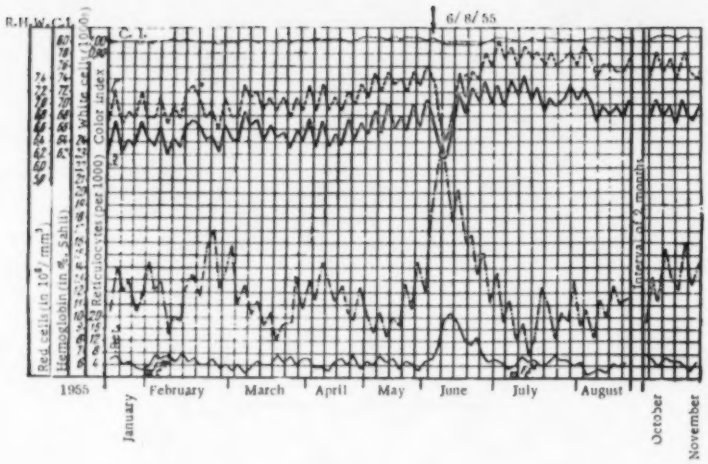

Fig. 1. Changes in the peripheral blood in the control dog Belyak. The arrow indicates the operation. The black squares denote times of radioactive iron administration.

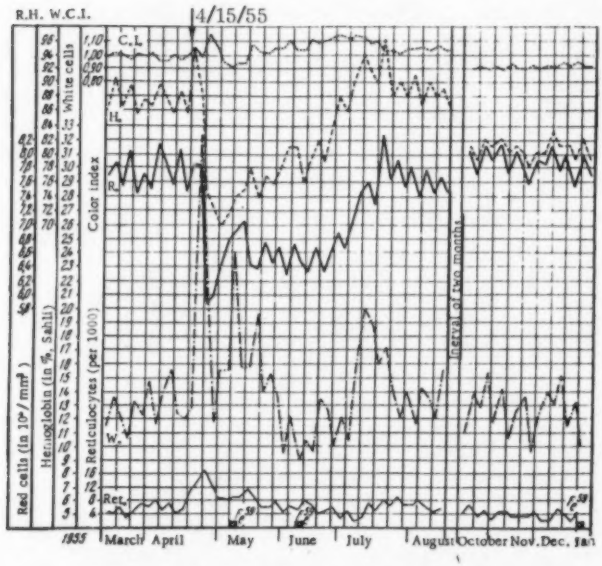

Fig. 2. Changes in the peripheral blood of the experimental dog Bars after subphrenic division of the vagus nerves.

The hemoglobin percentage rose rather more quickly, so that the color index was greater then unity. At this period the anemia was thus hyperchromic in character. The osmotic resistance of the red cells, especially the maximum value, increased appreciably. The mean red cell diameter increased by 0.8μ and a significant anisocytosis developed (Fig. 3). The findings of investigation of the bone marrow are shown in the table. They indicate that some degree of depression of erythropoiesis was observed at this period.

On two occasions, at the end of the first and second months after operation, this dog received radioactive iron (see Fig. 2). In contrast to the control animals the radioactivity of the plasma was reduced to one quarter, and on the following days no traces of Fe^{59} could be detected. Radioactivity was found in the red cells on the 3rd-5th day, reaching its maximum on the 6th-7th day, but its level was much lower. Despite the development of anemia, the utilization of radioactive iron in the experimental dog was thus sharply diminished by comparison with its utilization in the control dogs. It was found that 88% of the administered iron was excreted with the feces. At the end of the 3rd month the red cell count and the hemoglobin concentration had almost returned to their initial values. At this period the color index gradually fell. The changes in the osmotic resistance and diameter remained as before. The intensity of erythropoiesis was restored (see table).

Six months after operation the red cell count was at its initial level, but the hemoglobin concentration was reduced by 10-12% and the color index was now 0.9. The hemoglobin saturation index of the red cells, obtained by calculation [5], was considerably reduced (see Fig. 3). The osmotic resistance and diameter of the red cells remained as before.

In the course of the next three months the blood picture remained unchanged from that described. At the end of the investigation, radioactive iron was given on three occasions, and the same results were again obtained.

In the other two dogs, Tarzan and Fokus, the blood changes after operation were identical in character. Bilateral subphrenic division of the vagus nerves led to the development of a hyperchromic macrocytic anemia with signs of depression of erythropoiesis.

Six months after operation the red cell count had returned to its initial level, the intensity of erythropoiesis was restored and the anemia as such was corrected, although certain qualitative changes persisted. The decreased percentage of hemoglobin, the lowered hemoglobin saturation index of the red cells and the sharp fall in the utilization of radioactive iron evidently indicate some depression of the process of hemoglobinization of the red cells at a later period after operation.

According to findings obtained by V. A. Samtsov [7] and S. I. Yakovlev [9], after division of the vagus

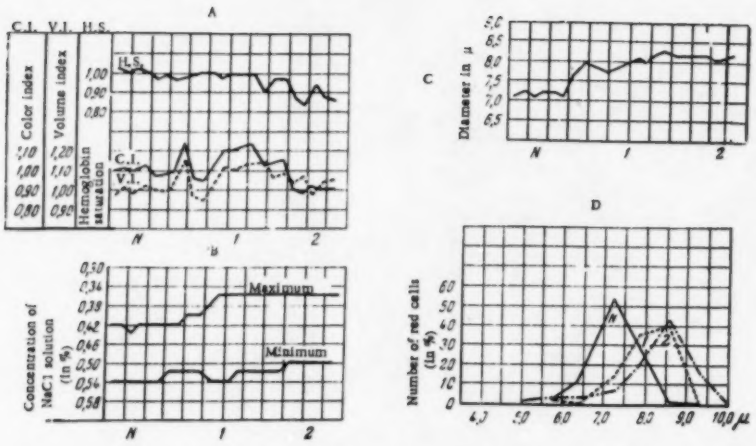

Fig. 3. Characteristic curves of the blood picture in the dog Bars after subphrenic division of the vagus nerves. A) Index of saturation of the red cells with hemoglobin (H.S.); B) osmotic resistance of the red cells (maximum and minimum); C) mean red cell diameter; D) erythrocytometric curve; N) before operation; 1,2) periods of anemia; V.I.) volume index.

Changes in Erythropoiesis in the Bone Marrow after Subphrenic Division of the Vagus Nerves in the Dogs Bars and Fokus

Periods of investigation	Date of investigation	Number of dividing forms (mitoses)	Erythroblasts	Pronormoblasts	Normoblasts: basophilic	Normoblasts: polychromatophilic	Normoblasts: orthochromatic	Reticulocytes	Red cell count in the peripheral blood (in 10^6/mm³)
		as a percentage of 100 bone marrow cells							
Bars									
Before operation	7/IV	0.1	0.5	1.8	5.7	10.3	15.2	0.7	7940
After division of the vagus nerves	5/27	0.3	0.9	2.4	4.3	8.7	11.9	0.7	6450
The same	7/27	0.2	0.6	2.1	5.4	10.4	14.7	1.1	7540
" "	12/24	0.1	0.6	1.7	6.1	10.1	15.5	0.8	7940
Fokus									
Before operation	4/7	—	0.7	1.7	6.2	11.6	14.7	0.5	7610
After division of the vagus nerves	5/6	0.3	1.1	2.5	4.7	9.9	12.9	2.1	6325
The same	7/26	0.1	0.6	1.9	5.7	11.2	15.2	0.7	6940
" "	7/21	0.2	0.8	1.8	6.4	11.9	15.4	0.5	7465

nerves in dogs, the antianemic activity of the gastric juice is sharply decreased. The morphological analysis which we undertook showed that after division of the vagus nerves the gastric mucosa undergoes a series of characteristic changes, testifying to disturbances of the nutrition of this area. The changes were seen especially clearly in the intramural nervous system of the stomach. Areas with complete degeneration of the intramural nerve cells were seen, in which only the cell membranes were preserved, here and there with pyknotic nuclei, waxy accumulations of myelin, and so on. Similar findings were published by E. A. Rudik [6].

So far as the blood system is concerned, the results of division of the vagus nerves was th. the development of a hyperchromic anemia, and —in the stomach itself — trophic disturbances and degeneration of the intramural nerve cells and apparatus. The latter may lead to a disturbance of the synthesis of antianemic factor in the gastric mucosa and may be the cause of development of the anemia which was observed.

In our opinion, however, the decrease in the antianemic activity of the gastric juice could hardly cause the development of anemia. Besides the stomach, the duodenum and small intestine are known to possess considerable antianemic activity. From the foregoing it may be postulated that production of antianemic factor is disturbed not only in the stomach, but also in the remaining part of the digestive tract.

Division of the vagus nerves involves destruction of both the efferent and afferent connections of the stomach with the central nervous system. The abolition of afferent impulses from such an extensive receptor region as the stomach may lead to failure of information and to disturbance of the central nervous mechanisms regulating the blood system. This explanation is based on V. N. Chernigovskii's hypothesis [8] of the great importance of constant tonic impulses, passing from the internal organs to the central nervous system, in the maintenance of the constancy of the blood system.

The disturbances which we observed are, of course, functional in character. The diminished red cell count is gradually restored and the anemia as a rule is corrected.

The sharp disturbance of utilization of radioactive iron, and the slight lowering of the hemoglobin concentration and the hemoglobin saturation index of the red cells however, are evidence that qualitatively more delicate and complicated processes such as hemoglobinization of the red cells suffer more from denervation than do quantitative processes, which are more independent, such as the proliferation and development of erythroid tissue. In this connection it may also be suggested that the cessation of afferent impulses from the stomach to the central nervous system leads not only to disturbance of efferent influences on the processes of synthesis of the antianemic factor in the alimentary tract, but also to disturbance of direct efferent influences on the bone marrow tissue.

SUMMARY

The author studied the effect of bilateral subdiaphragmatic division of the vagus nerves on the blood system in chronic experiments on dogs.

The development of hyperchromic macrocytic anemia lasting for a period of 3 months was noted. Sharp reduction in the utilization of radioactive iron (Fe^{59}) was found together with degenerative and trophic disturbances in the gastric mucosa. Later, the red cells recovered their normal level.

A relatively low hemoglobin percentage and disturbed radioactive iron utilization lasted up to the end of the investigation (10 months). Some mechanisms of disturbances observed in the blood system are discussed.

LITERATURE CITED

[1] V. G. Vogralik, The Work of Russian Scientists on the Nervous Regulation of the Blood System (Gor'kii, 1953) [In Russi].

[2] D. I. Gol'dberg, Essays in Hematology (Tomsk, 1952) [In Russian].

[3] N. S. Dzhavadyan, Experimental Data on the Nervous Regulation of the Blood System. Doctorate dissertation (Moscow, 1954) [In Russian].

[4] R. A. Durinyan, Experimental Analysis of Anemias Caused by Denervation of Certain Internal Organs. Candidate's dissertation (Moscow, 1956) [In Russian].

[5] I. A. Kassirskii and G. A. Alekseev, Clinical Hematology [Moscow, 1955) [In Russian].

[6] E. A. Rudik, Arkh. Patol. 6, 59 (1952).

[7] V. A. Samtsov, Arkh. Patol 17, 1, 22 (1955).

[8] V. N. Chernigovskii and A. Ya. Yaroshevskii. The Nervous Regulation of the Blood System (Moscow, 1953) [In Russian].

[9] S. I. Yakovlev, The Role and Importance of the Nervous System and the Vitamin B Complex in Erythropoiesis. Doctorate dissertation (Leningrad, 1955) [In Russian].

[10] E. Komiya, Diezentralnervose Regulation des Blutbildes (Stuttgart, 1956).

HEMOPOIETIC PROPERTIES OF THE SERA OF HEALTHY AND ANEMIC ANIMALS (CATS)

E. L. Kan

Laboratory of Experimental Pathology and Therapy (Head—G. S. Kan) of the Leningrad Institute of Tuberculosis (Director—Prof. A. D. Semenov), and the Group Directed by Active Member AMN SSSR M. D. Tushinskii

(Presented by Active Member AMN SSSR M. D. Tushinskii)

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The humoral mechanisms of regulation of the blood system have been the subject of many hematological investigations in recent years. The role of hemopoietins in the maintenance of the constancy of the composition of the blood and in increasing the cell content in certain anemias has been fully investigated and discussed. This trend in hematology was initiated by the researches of Carnot and Deflandre [9]. These and many other workers [12,14,15] showed that the serum of healthy animals and of animals subjected to bleeding, and also the serum of animals with experimental phenylhydrazine anemia, like the serum of patients with certain forms of anemia, when injected into a recipient animal, causes stimulation of erythropoiesis in the latter.

Although much work has been done on the study of the role of hemopoietins, much remains unclear and unsolved. The presence of hemopoietins in the serum of healthy animals, for instance, has not yet been demonstrated convincingly. The question of the relationships between the hemopoietins and nervous mechanisms has also to be considered.

For a number of years we have investigated the role of the nervous system in the regulation of the blood system [4,5]. In connection with the development of our knowledge of the hemopoietins, it became necessary to ascertain the role and the place of this factor in the changes taking place in the blood system under the influence of certain procedures which we carried out on the nervous system.

In the present communication we describe the results of a study of the hemopoietic properties of the blood of cats during the various phases of development of an experimental hypochromic anemia in these animals after denervation of the spleen.

In our previous investigations [4,5] we showed that division of the splenic nerves leads regularly to the development of a hypochromic anemia with disturbances of erythropoiesis, and this has been confirmed by other authors [1,2,3,6]. In view of the fact that the problem of the hemopoietic properties of the serum of healthy cats, on which the main experiments were carried out, has not been discussed in the literature, a study was made of this problem also.

METHOD

Blood was taken from experimental donor cats, under anesthesia, from the femoral and splenic veins and the carotid artery. Serum was obtained from the blood by centrifugation, and was injected subcutaneously, in volume of 3-9 ml, into healthy recipient rabbits. The test serum of each experimental donor cat was usually injected into two rabbits. For 2-3 weeks before injection of the serum, the red cell count, the hemoglobin concentration, the reticulocyte count, and the composition of the bone marrow were determined in the rabbits. After injection of the serum, the blood was investigated every day for the first week, on alternate days during the second week, and thereafter once every 5 days for one month. In all, the properties of the sera of 10 healthy cats and 23 cats with experimental anemias were investigated. Test serum was injected into 73 rabbits.

In a proportion of cases a nonprotein extract of the plasma was injected, prepared by Borsuk's method [8]. Blood was obtained from anesthetized cats after intravenous injection of liquid heparin in a dose of 0.2 ml per animal, from the carotid artery, and centrifuged. The pH of the plasma was adjusted to 5.5 with N HCl. The plasma was then boiled for 10-15 minutes, after which it was filtered. The filterate thus obtained was made up to the original volume with distilled water and injected subcutaneously into recipient rabbits in a dose of 10-15 ml daily for 3-4 days.

Experimental neurogenic anemia was produced by denervation of the spleen, the operative conditions being the same as those previously described [4].

Posthemorrhagic anemia was produced by bleeding from the femoral vein: 60-70 ml of blood was removed through a tied-in cannula. The hemopoietic properties of the serum of these animals were studied 24 hours after this procedure.

RESULTS

In the first series of experiments we studied the hemopoietic properties of the serum of healthy cats. In 12 of 15 cases, this serum caused slight and irregular fluctuations in the recipient rabbits, affecting the red cell count and the hemoglobin concentration, which increased and decreased without transgressing the limits of the "spontaneous" variations in the composition of the blood. The red cell count rose by 380,000-1,350,000/mm^3 (8-29%), with an average increase of 550,000/mm^3 (11%). The red cell count decreased by 320,000-1,850,000/mm^3 (5-25%), with an average fall of 740,000/mm^3 (14%). The average increase in the hemoglobin concentration was 7.1 units on the Sahli scale, the average decrease 5.8 units. In all the experiments, as in our previous observations, no parallel trend could be discerned between the changes in the red cells and hemoglobin.

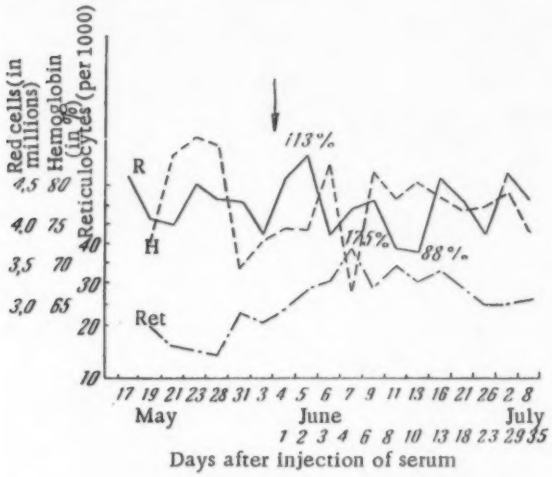

Fig. 1. Changes in the red cell count (R), hemoglobin concentration (H), and reticulocyte count (Ret) in a rabbit in response to the injection of 3 ml of serum from a healthy cat. The arrow (↓) indicates the day on which the serum was injected.

In 14 of 15 experiments, the serum of healthy cats caused an increase in the reticulocyte count regularly in the recipient rabbits. The average increase was 16 per 1000 (92% in relation to the original level). An increase in the reticulocyte count was observed starting with the 3rd-4th day after injection of the serum, and the maximum reticulocytosis was found on the average on the 8th-10th day (Fig. 1).

Apart from a moderate reticulocytosis, no other marked changes of erythropoiesis were present in the bone marrow.

Injection of physiological saline into 8 control rabbits caused no changes in the composition of the blood or bone marrow in any experiment that exceeded the limits of "spontaneous" variations.

In the second series of experiments we investigated the hemopoietic properties of the blood of experimental

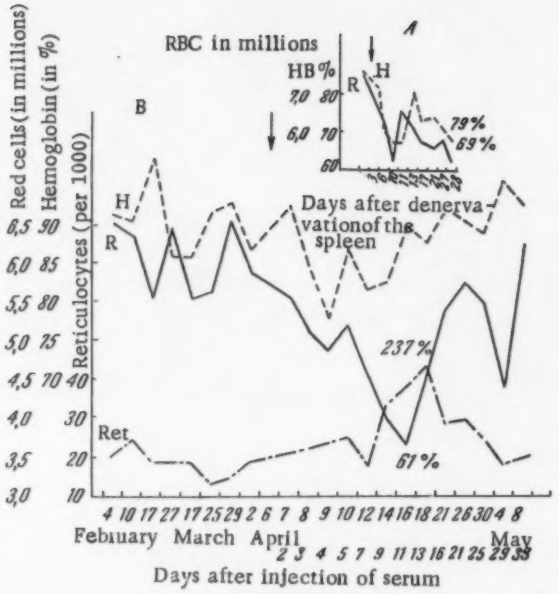

Fig. 2. A) Changes in the red cell count (R), and hemoglobin concentration (H) in a cat after denervation of the spleen just before the time of taking the blood. The arrow (↓) denotes the time of denervation. B) Changes in the red cell count (R), hemoglobin (H), and reticulocyte count (Ret) in a rabbit in response to the injection of 3 ml of serum from a cat at the height of development of anemia, on the 28th day after denervation of the spleen (the red cell count fell to 2,880,000 or 69% of the initial value—A). The arrow (↓) denotes the day the serum was injected.

cats from 4 to 66 days after the operation of division of the splenic nerves, when anemia had developed, and also at later periods, when the initial composition of the blood had been restored. In all 36 experiments, within a few days of the injection of "anemic" donors' serum, the recipient rabbits developed a regular and pronounced fall in the red cell count (by 270,000-3,320,000/mm^3). On the average, the red cell count fell by 1,250,000/mm^3, or by 22% in relation to the original level. The increase in the red cell count was irregular and did not exceed 9%, i.e. the percentage of error.

The hemoglobin concentration rose on the average by 7.1 and fell by 8.1 Sahli units. The fall in the hemoglobin concentration was 1.5 times greater than the fall in the control experiments (Fig. 2).

Injection of serum from "anemic" donor cats into rabbits, in 35 of the 36 experiments caused an increase in the reticulocyte count (by 7-39 per 1000). The reticulocyte count rose on the average by 13.2 per 1000 or by 89% by comparison with the original level. The maximum reticulocytosis in these experiments took place on the 8th day from the moment of injection of the serum. The increase observed in the reticulocyte count following injection of "anemic" serum was slightly smaller than that after injection of the serum of healthy cats.

TABLE 1. Correlation between the Degree of Anemia in the Donor (Cat) and the Degree of the Changes in the Composition of the Recipient's (Rabbit) Blood

Cats		Rabbits				
Day after denervation	Mean fall in the red cell count (in millions)	Day of maximum fall in the red cell count	Mean fall in the red cell count (in millions)	Day of maximum increase in the red cell count	Mean increase in the red cell count (in millions)	Mean increase in the reticulocyte count (per 1000)
4-10th	2.18	20th	0.63	3rd	0.51	17.1
11-20th	1.86	13th	0.98	8th	0.42	18
21-30th	2.13	10th	1.65	8th	0.43	15
31-40th	1.81	16th	1.27	2nd	0.54	16

TABLE 2. Mean Results of Experiments to Study the Hemopoietic Properties of the Blood of Healthy and Anemic Cats

Indices of the composition of the recipients blood / Characteristics of donor's serum	Red cells				Hemoglobin		Reticulocytes	
	increase		decrease		increase	decrease	increase	
	in millions	as % of original value	in millions	as % of original value	Sahli units		per 1000	as % of original value
Serum in splenogenic anemia	0.48	9	1.25	23	7.1	8.1	13.2	89
Nonprotein extract of plasma in splenogenic anemia	0.56	8	1.06	22	7.3	8.0	23.1	140
Serum in period of compensation of splenogenic anemia	0.82	13	0.71	13	11.5	5.3	12.2	74
Serum of healthy cats	0.55	11	0.74	14	7.2	5.8	16.1	92
Serum in posthemorrhagic anemia	0.76	12	0.27	5	8.3	3.2	19	103

The fall in the red cell count in the peripheral blood of the rabbits which resulted from injection of the test serum was to some degree due to disturbance of erythropoiesis. This occurred in 11 of the 12 experiments in which the changes in hemopoiesis in the bone marrow were studied. In the first period after injection of the serum there were signs of stimulation of erythroblastic maturation, associated with an increase in the reticulocyte count; subsequently, in the 2nd-3rd week, stimulation of erythropoiesis was replaced by depression, with a fall in the number of reticulocytes. The total number of erythroblasts fell by 4-20.2%.

Analysis of the results obtained also showed that a correlation was present between the depth of anemia in the donors and the severity of the changes in the composition of the blood and in hemopoiesis in the bone marrow in the recipient. Furthermore, it was found that the serum did not affect the red cell composition of the blood of the rabbits to an equal extent at all periods after denervation (Table 1). In the period of maximum anemia in the experimental cats, observed on the 21st-30th day after denervation of the spleen, the serum of these animals evoked the most pronounced fall in the red cell count and hemoglobin concentration and the smallest rise in the reticulocyte count. Another important fact was that the changes listed above in the blood system of the rabbits took place soonest after the injection of the "anemic" serum.

In the period of restoration of the composition of the blood in the cats suffereing from anemia after denervation of the spleen, their serum caused changes in the composition of the blood of the recipient rabbits similar to those observed in control experiments.

From the results obtained, the question arose at to the nature of the humoral factor present in the blood at the height of development of anemia after denervation of the spleen. Some authors [11,15] associate the hemopoietins with the protein fractions of the blood; others [8,10] deny that they are blood proteins.

In order to study thsi problem, and to exclude the possibly toxic influence of the foreign serum, a third series of experiments was performed in which a nonprotein extract of plasma was injected. In a volume of 23-45 ml, nonprotein plasma extract, taken at the height

of theanemia, caused the same reaction in principle in the rabbits as did the whole serum or plasma under the same experimental conditions. The degree of severity of the reaction, however, was different: In all 8 experiments, a less pronounced fall in the red cell count and a more marked reticulocytosis were observed that after the injection of whole serum (Table 2). On the average, the red cell count in these experiments fell by 1,060,000 (by 22%) and the reticulocyte count rose by 23.1 per 1000 (by 140% compared with the initial level). Just as after the injection of serum, the bone marrow showed signs of disturbance of erythropoiesis. It was thus established that a nonprotein extract of the plasma also possesses the property of producing anemia. At the same time it must be emphasized that the last series of experiments showed that the factor found in the serum in neurogenic anemia was to some extent associated with the proteins of the blood.

In a fourth series of experiments we showed that the serum of cats taken 24 hours after acute loss of blood caused a reaction in recipient rabbits which, in principle, was of quite a different type from that due to the serum of cats with neurogenic anemia: An increase in the red cell count (on the average by 760,000/mm^3, or 12%) and an insignificant fall in this count (on the average by 270,000/mm^3, or 5%), and also a more marked reticulocytosis, which was observed sooner—on the 5th day after injection of the serum (Fig. 3 and Table 2). The reticulocyte count rose on the average by 19 per 1000 (by 103%). The results of these last experiments thus showed that

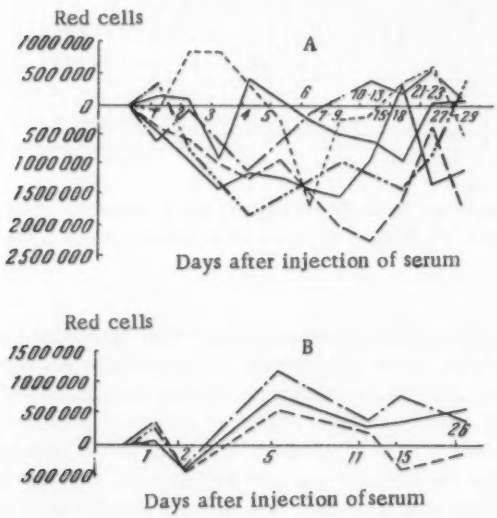

Fig. 3. A) Changes in the red cell count in 6 rabbits in response to the injection of 3 ml of serum from cats in the period of maximum development of anemia casued by denervation of the spleen. B) Changes in the red cell count in 3 rabbits in response to the injection of 3 ml of serum taken from cats 24 hours after acute loss of blood.

the serum of cats in certain anemic states could have a stimulating, hemopoietic effect on erythropoiesis.

We must now consider the cause of the fall in the red cell count of the recipient rabbits in response to the injection into these animals of the serum of cats in the phase of development of anemia due to denervation of the spleen. It is possible that the foreign cats' serum causes hemolysis in rabbits, and anemia develops as a result of this process. While we cannot altogether dismiss this mechanism, we cannot regard it as the most important, for there is indirect evidence against the occurence of hemolysis. The increase in the reticulocyte count, for instance, was 1.5 times greater after injection of the nonprotein plasma extract than after injection of serum (see Table 2). Furthermore, the increase in the reticulocyte count itself in response to injection of "anemic" serum was smaller than that after injection of serum from healthy animals. It could be assumed that cats' serum, whatever the form of anemia, would possess the property of causing anemia when injected into other animals. The results obtained, however weigh against this hypothesis: The serum in posthemorrhagic anemia possesses hemopoietic properties and causes stimulation of erythropoiesis in recipients. We must therefore consider that after denervation of the spleen, substances are formed which possess the power to interfere with erythropoiesis. The nature of the factor thus found is not yet known. It may be a factor of the erythrodieretin type [7], or it may possibly be the factor described in the literature under the name of "Anamie-stoff" [13].

SUMMARY

It was demonstrated experimentally that the subcutaneous injection of sera (3-9 ml) obtained from healthy cats into recipient rabbits causes moderately marked fluctuations in the red cell count and hemoglobin level and reticulocyte count. At the height of anemias after splenic denervation both the serum and plasma contain an anemic factor which decreases the red cell count and hemoglobin level when injected into other animals and disturbs erythroblast maturation with subsequent inhibition of erythropoiesis. In similar experiments the nonprotein plasma extract causes analogous, although less pronounced changes. The serum in posthemorrhagic anemia possesses hemopoietic activity and causes moderate polyglobulonemia and pronounced reticulocytosis in recipient rabbits.

LITERATURE CITED

[1] N. N. Beller, Participation of the Nervous System in the Regulation of the Composition of the Blood and Oxygen Saturation of the Arterial Blood during Anoxia. Author's summary of dissertation (Leningrad, 1957) [In Russian].

[2] R. A.Durinyan, Experimental Analysis of Anemias Caused by Denervation of Certain Internal Organs. Author's summary of dissertation [Moscow, 1956) [In Russian].

[3] M. A. Kalnyn', The Effect of Denervation of the Spleen on Hemopoietic Processes. Author's summary of dissertation (Riga, 1955) [In Russian].

[4] E. L. Kan, Data from a Study of the Effect of the Nervous System on the Composition of the Blood. Author's summary of dissertation (Leningrad, 1953) [In Russian].

[5] E. L. Kan and G.I. Vavilin, Arkh. Patol 2, 61 (1957).

[6] N. S. Rozanova and E. A. Zhukova, Problemy Gematol. 3, 14 (1956).

[7] Ya. G. Uzhanskii, Uspekhi Sovermennoi Biol. 22, 1, 63 (1946).

[8] H. Borsook, Blood 9, 734 (1954).

[9] P. Carnot and C. Deflandre, Compt. rend. Acad. Sci. 143, 384 (1906).

[10] A. N. Contopoulos, R. McComps, J. H. Lawerence et al., Blood 12, 614 (1957).

[11] G. Hodson and I. Toha, Blood 9, 299 (1954).

[12] G. Hodson and I. Toha, Blood 179, 633 (1957).

[13] E. Komja, Die Zentralnervose Regulation des Blutbildes (Stuttgart, 1956).

[14] D. R. Korst and F. H. Betheli, Clin. Res. Proc. 5, 142 (1957).

[15] W. A. Rambach, A. L. Alt, and J.A. D. Cooper, Blood 12, 1101-1113 (1957).

[16] F. Stohlman and G. Brecher, Proc. Soc. Exper. Biol. Med. 91, 1 (1956).

CHANGES IN THE PERIPHERAL BLOOD AND BONE MARROW OF RABBITS IN EXPERIMENTAL EPILEPSY

V. M. Karlinskii

Department of Pathological Physiology (Head—Prof. Ya. A. Lazaris), Karaganda State Medical Institute

(Presented by Active Member AMN SSSR V. V. Parin)

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During an investigation of the blood of patients with epilepsy we found [3] that a true leucocytosis and an increase in the platelet count took place under the influence of epileptic fits. The functional state of the bone marrow was characterized by the presence of a myeloid reaction with an increase in the content of myeloblasts, promyelocytes, neutrophilic myelocytes and metamyelocytes, and also an increase in the number of megakaryocytes. We regarded the changes in the bone marrow as the result of changes accumulating gradually in connection with the epileptic fits.

In order to ascertain the truth of this hypothesis, we studied the peripheral blood and the bone marrow during experimental epilepsy in rabbits.

Several authors have studied the effect of experimentally induced epilepsy on the composition of the peripheral blood. I. N. Davydov [1], who produced epileptic fits in rabbits and cats by electrical stimulation of the brain, observed an obvious leukocytosis in the peripheral blood. The increase in the white cell count took place on account of an increase in the absolute and relative number of lymphocytes, a relative decrease and an absolute increase in the number of neutrophils, and an increase in the number of monocytes. This author considers that the postparoxysmal leukocytosis is redistributive in nature, and that contraction of the spleen plays an essential role in its formation. In more prolonged investigations on rabbits, N. M. Eremeeva [2], who used electrical stimulation of the brain, observed an obvious leukocytosis within a few minutes of the action of the current, reaching its maximum after 1-2 or, occasionally, 3 hours. The leukocytosis developed on account of neutrophils, lymphocytes, and monocytes. Young forms of neutrophils sometimes appeared in the peripheral blood. Swensson [4] described the development of leukocytosis in rats 2-3 minutes after receiving injections of convulsive doses of cardiazol. We do not know of any reports on the state of hemopoiesis in the bone marrow during experimental epilepsy.

METHOD

Experiments were carried out on 16 rabbits of both sexes, weighing 1.2-2.2 kg. In order to produce fits, a 10% solution of cardiazol was injected intravenously in a dose of 0.3 ml/kg body weight. Fits were caused repeatedly at intervals of 1-3 days. Blood for examination was taken from the veins of the ear before the fit, immediately after the fit, and 15 minutes, 1 hour, 2 hours, 3 hours, and, in some experiments, 12 hours after the fit. Determinations were made of the hemoglobin concentration; the red cell, platelet, and white cell counts; and the white cell formula. Bone marrow was extracted by puncture of the ischial tuberosity before and after 8-33 fits.

RESULTS

Immediately after injection of cardiazol a fit began. Three periods could be distinguished in its development: A very short (2-12 seconds) period of major clonic convulsions, a period of tonic convulsions (12-25 seconds), and a period of medium and minor clonic convulsions, lasting from 29 seconds to 79 minutes.

In the course of the fit, all the experimental rabbits showed slight changes in the hemoglobin concentration and red cell count, which lay within the limits of error of the method. With regard to the numbers and composition of the white cells, regular changes were observed in all 16 rabbits. As a rule, after a fit, the white cell count was increased. The maximum rise in the white cell count took place in 8 rabbits immediately after the fit, in 5 rabbits 15 minutes after, and in 3 rabbits 1 hour after the fit. At the moment of maximum rise, the white cell count amounte to 150-244% of its initial value. The white cell count then gradually fell, after varying intervals of time (after 1 hour in 6 rabbits, after 3 hours in 6 rabbits, and after 12 hours in 3 rabbits). Typical changes in the number and composition of the white cells as a result of a cardiazol convulsion are shown in Table 1.

The level of the white cell count depended on the duration and severity of the convulsions. The maximum elevation of the white cell count over the original level (225-244%) was observed 4 times after long fits with severe convulsions.

Along with the changes in the white cell count, regular changes were observed in the white cell formula.

TABLE 1. Changes in the Number and Composition of White Cells in Rabbit No. 4 under the Influence of a Fit

Name of cells	Before fit	Immediately after fit	At an interval after the fit of			
			15 min	1 hr	2 hr	3 hr
White cells	6750	9800	15,000	9850	8300	7100
Basophils, in %	—	3.0	1.0	4.0	—	1.5
Eosinophils, in %	1.0	—	—	—	1.0	0.5
Pseudoeosinophils, juvenile, in %	—	—	1.0	1.5	1.5	—
Pseudoeosinophils (stab cells), in %	1.0	2.5	4.5	4.5	8.5	2.0
Pseudoeosinophils, segmented, in %	35.0	18.5	26.5	57.5	68.5	63.5
Lymphocytes, in %	57.5	61.0	64.5	28.5	18.5	25.5
Monocytes, in %	5.0	5.0	2.5	4.0	2.0	7.0

TABLE 2. Changes in the Bone Marrow in Rabbit No. 12 Under the Influence of Cardiazol Convulsions

Name of cells	Before experiment	After 21 fits	Name of cells	Before experiment	After 21 fits
	in %			in %	
Hemocytoblasts	—	0.4	Monocytes	—	—
Myeloblasts	1.4	2.2	Reticuloendothelial cells	1.6	1.8
Promyelocytes	1.8	3.4	Ferrat cells	0.2	1.0
Pseudoeosinophilic myelocytes	7.4	8.6	Plasma cells	0.2	1.0
Eosinophilic myelocytes	0.4	0.4	Botkin-Gumprecht cells	0.8	1.6
Basophilic myelocytes	—	0.2	Megakaryocytes	0.2	0.4
Pseudoeosinophilic metamyelocytes	8.2	9.4	Proerythroblasts	1.8	2.2
Eosinophilic metamyelocytes	—	0.2	Basophilic erythroblasts	5.0	5.6
Pseudoeosinophilic stab cells	7.4	10.0	Polychromatophilic erythroblasts	22.2	20.2
Eosinophilic stab cells	0.2	—	Oxyphilic erythroblasts	19.0	14.2
Pseudoeosinophilic segmented cells	13.8	17.4	Bare nuclei of erythroblasts	1.8	1.8
Eosinophilic segmented cells	0.2	0.4	Destroyed cells	0.8	1.4
Adult basophils	1.2	2.0	Dividing cells	0.8	1.4
Lymphocytes	3.2	2.3	Index of maturation of pseudoeosinophils	0.8	1.8
			Ratio between leukopoietic and erythropoietic cells	0.8	1.2

The degree of the shift also depended on the duration and, still more, the severity, of the fit.

In the first few minutes after the fit, the absolute number of lymphocytes was increased, but it then began to diminish. After 15 minutes to 1 hour pseudoeosinophils were predominant in the formula, varying in number from 39% to 82.5%. As the white cell count fell, the number of pseudoeosinophils did not diminish but, on the contrary, showed a tendency to increase. The absolute number of pseudoeosinophils remained at a high value when the total white cell count had returned to its original level.

In every case an increase in the pseudoeosinophilic stab cells (4-12.5%) was observed after injection of cardiazol, and in 11 animals 0.5-2.5% of juvenile pseudoeosinophils appeared at the height of the changes in the white cell formula. Eosinophils disappeared from the blood and the number of basophils and monocytes increased.

In 14 rabbits the platelet count was raised after the fits. The highest rise took place in the first 15 minutes after the fit, and amounted to 123-232% by comparison with the original value. Immediately after the rise in the platelet count it began to fall gradually, but in 9

cases the platelet count was still above its initial level 2-3 hours after the end of the fit.

In order to exclude any toxic action of cardiazol on the composition of the blood, in 6 experiments the drug was injected in doses too small to cause convulsions, but very close to the convulsive dose. Blood was taken at the same times as after the epileptiform attack. Under these circumstances no detectable changes took place in the number and composition of the white cells and in the platelet count.

In order to ascertain the cause of the changes in the peripheral blood, in 11 rabbits the effect of repeated convulsive fits on the functional state of the bone marrow was studied. Repeated epileptiform attacks gave rise to a myeloid reaction, which affected the granulopoietic and megakaryocytic series. As an example we describe the myelogram of rabbit No. 12 (Table 2).

The ratio between leukopoietic and erythropoietic cells was increased on account of an increase in the number of leukopoietic cells. An increase was observed in the number of myeloblasts, promyelocytes, pseudoeosinophilic myelocytes, and metamyelocytes. Whereas before the fits, their numbers varied between 3.4 and 31%, after the fits they increased to 15.3-38.8%. An increase also took place in platelet-forming megakaryocytes. We found no regular changes in erythropoiesis.

As a result of a fit, considerable changes thus took place in the morphological composition of the peripheral blood, mainly affecting the number and composition of the white cells and the platelet count. The leukocytic reaction in the first few minutes evidently arose from a redistribution of the blood. This was shown by the increase in the number of lymphocytes observed in the first minutes after the fit. The further increase in the white cell count resulted from the higher rate of liberation of adult pseudoeosinophils from the bone marrow. This was demonstrated by the shift of the pseudoeosinophils to the left, with the appearance of even juvenile forms in the peripheral blood. The true character of the resulting leukocytosis was confirmed by the onset of changes in the functional composition of the bone marrow, in the form of stimulation of granulopoiesis. The increased number of platelets in the peripheral blood after the epileptiform fit was explained by a study of the bone marrow in which an increase in the platelet-forming megakaryocytes was found.

From a comparison of the results of the investigation of hemopoiesis in experimental cardiazol epilepsy and clinical epilepsy in man, it may be concluded that the changes in hemopoiesis in both cases are closely similar.

From our findings we have reason to consider that the changes in hemopoiesis in epilepsy are connected with the fits. These changes may last much longer than the fits themselves, and may gradually become intensified.

SUMMARY

The effect of experimentally induced cardiazol epilepsy on the peripheral blood and bone marrow was studied in 16 rabbits. The epileptiform attacks provoked leukocytosis and a rise of the red cell count. The leukocytic reaction appeared during the first few minutes of an increased number of lymphocytes, and later on of pseudoeosinophils. Following several attacks, an intensified granulopoiesis and an increased number of platelet producing forms of megakaryocytes were observed in the bone marrow.

There were no modifications in the red blood picture.

The author concludes that the changes occuring in the blood are connected with the intensified bone marrow function, under the effect of epilepitform fits.

LITERATURE CITED

[1] I. N. Davydov, Byull. Éksptl. Biol. i Med. 11, 6, 557 (1941).

[2] N. M. Eremeeva, Collected Transactions of the Kursk Medical Institute 2, 15 (1955) [In Russian].

[3] B. M. Karlinskii, Trudy Karagandinskogo Med. Inst. 1, 396 (1957).

[4] A. Swensson, Ztschr. ges. exper. Med. 122, 14 (1953).

THE NATURE OF SPINAL SHOCK
COMMUNICATION IV. ATONY OF THE MOTOR NEURON IN SPINAL SHOCK

G. N. Sorokhtin, O. P. Minut-Sorokhtina, and Yu. B. Temper

Department of Physiology (Head—Prof. G. N. Sorokhtin), Khabarovsk Medical Institute

(Presented by Active Member AMN SSSR V. V. Parin)

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In research carried out in 1945 [1], we regarded spinal shock as a syndrome of atony of the motor neuron, although we had no direct proof of the localization of the developing atony.

In our third communication we showed that the sensory links of the reflex arcs of the spinal cord play no part in the development of spinal shock. The quantitative and qualitiative characteristics of the volleys of impulses produced by standard stimulation of the receptors of the skin were not altered by cordotomy over the whole extent of the afferent conducting pathways of the spinal cord.

In the present investigation the problem of the site of development of atony in the spinal reflex arc was investigated by the study of myotatic reflexes caused by stretching of a muscle or by electrical stimulation of the posterior root of the spinal cord.

METHOD

In the first series of experiments we recorded the action potentials of the gastrocnemius muscle of the frog (Rana chensinensis) during reflex contractions of this muscle in response to its stretching. Ths hip and knee joints of the limb under study were firmly fixed, all the muscles with the exception of that being studied were denervated, and the skin of the limb was removed. Dorsiflexion of the foot was carried out by a standard method using pulleys, with a constant angle of stretching and with equal velocity. The electromyogram was recorded with the film moving at a speed of 25 mm/sec, with paired electrodes, 30 μ in diameter, inserted into the muscle and with no insulation in cross section.

In a second series of experiments, in order to eliminate the monosynaptic impulses, the tracing was made at a higher speed of movement of the photographic film (250 mm/sec). The potentials were recorded from the central end of the anterior root of the IXth nerve during stimulation of the posterior of the same nerve with closure of a constant current. All the anterior roots innervating the limb under study were divided.

The potentials were recorded on a two-channel cathod oscillograph.

RESULTS

Conventionally, in the first series of experiments the electromyogram reproduced the myotatic reflex of its own particular level. During stretching of the gastrocnemius muscle (Fig. 1,A,B,C), in the initial state the volley of monosynaptic and polysynaptic impulses was interrupted by a period of silence. This reflex pause period [4], of differing duration and depth, is characterized by anelectrontonic hyperpolarizational inhibition, arising in the motor neuron in response to recurrent impulses [3,5,6].

In the experiments in which a state of increased excitation followed immediately after cordotomy (Fig. 1,D), the period of reflex silence disappeared, and in its place there was a continuous, intensive volley, with considerable after-action.

In the other experiments in which spinal shock was developing, we were able to discern three types of reaction: complete disappearance of monosynaptic and polysynaptic impulses (Fig. 1,A), disappearance of the initial impulses with the preservation of a short volley on the cessation of stretching (Fig. 1,B), and finally, the development of reflex anelectrotonic inhibition of the period of silence (Fig. 1,C), becoming more intensive later on (the third segment of the electromyogram) until the retention of one single initial monosynaptic potential.

After 4-6 minutes, when spinal shock had passed over, the initial electromyogram was restored (Fig. 1,B). During the change over from spinal shock to the state of increased excitation, the electromyogram showed greater activity than was observed before cordotomy (third cut on Fig. 1, A, and fourth cut on Fig. 1, C).

The disappearance of the potentials of the myotatic reflex showed convincingly that the genesis of spinal shock was predetermined by the motor neuron cell, and individual experiments testifying to the intensification of the reflex period of silence (Fig. 1,C) suggest the hyperpolarization (anelectrotonic state) of the motor neuron in the period of spinal shock.

In the second series of experiments we examined the fate of the initial monosynaptic potentials in the

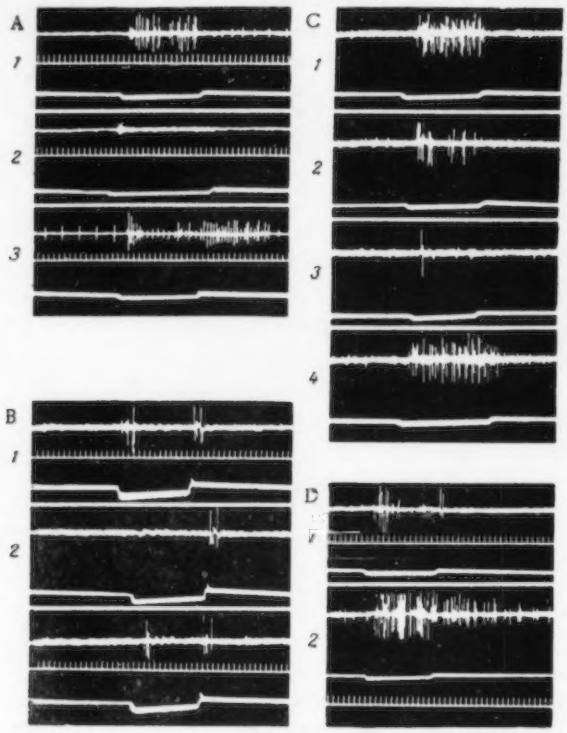

Fig. 1. Electromyogram of the gastrocnemius muscle. (First series of experiments: No. 30—A; 28—B, 16—C and 31—D). 1) Before cordotomy; 2) immediately after cordotomy; 3 and 4) a few minutes after cordotomy. In each cut, the top line represents the oscillogram and the bottom line the stimulation marker (stretching the muscle). Time marker—50 millisec.

anterior root in response to electrical stimulation posterior root of the same segment (the IXth) on the same side. In experiment No. 30(Fig. 2,A), after cordotomy, when spinal shock was developing, the monosynaptic potentials disappeared completely and were reestablished after 6 minutes, with a considerable shortening of the duration of central inhibition. In experiments Nos. 36(Fig. 2,B) and 2(Fig. 2,C) the spinal shock was characterized by a considerable decrease in the monosynaptic potential, which rose considerably in the period of increased excitation of the spinal reflexes and appeared sooner in time than in the original state before cordotomy.

Both series of experiments described in the present communication show that the link responsible for the development of spinal shock is the motor neuron cell, which as the final common spinal path, becomes passively atonic when deprived of supraspinal impulses. The state of atony of the motor neuron cell is shown by the fact that its polarization is increased – an anelectrotonic state develops, which diminishes it preparedness for non-incremental excitation.

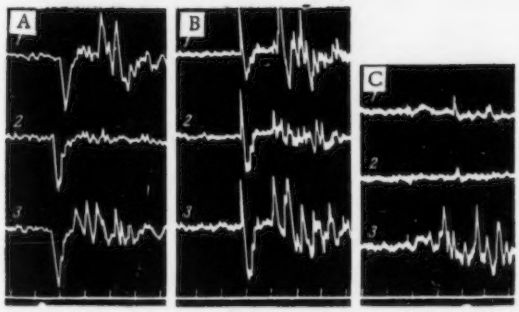

Fig. 2. Potentials of the anterior root. (Experiments of the second series: No. 30—A; 36—B and 2—C). 1) Before cordotomy; 2) immediately after cordotomy; 3) a few minutes after cordotomy. Stimulation of the posterior root is indicated by an artefact on the oscillogram. Time marker—10 millisec.

SUMMARY

The condition of motorneurons was studied during spinal shock in experiments on frogs. The myotatic reflex of the gastrocnemius muscle was recorded by electromyograms in the first series of experiments. The disappearance of the initial impulses or an increase of the anelectrotonic "silence period" was noted in the monosynaptic and polysynaptic impulses in spinal shock. In the second series of experiments, the authors recorded the potentials of the IXth anterior root during electric stimulation of the corresponding posterior root. The disappearance or reduction of the intial monosynaptic potential following chordotomy, and its restoration after recovery from spinal shock, pointed to the fact that the motorneurons cells are the site of the spinal shock development. The anelectrotonic condition of the motorneuron during the shock period is an atonia provoked by the exclusion of the supraspinal impulses.

LITERATURE CITED

[1] G. N. Sorokhtin, Byull. Éksptl. Biol. i Med. 19, 3, 73 (1945).

[2] G. N. Sorokhtin, O. P. Minut-Sorokhtina and Yu. B. Temper, "Communication III", Byull. Éskptl. Biol. i Med. 9, 16 (1959).*

[3] D. F. Denny-Brown, Proc. Roy. Soc. Ser. B 103, 321 (London, 1928).

[4] J. C. Eccles, P. Fatt and K. Koketsu, J. Physiol. 126, 524 (1954).

[5] R.Granit, Receptors and Sensory Perception (London, 1955).

[6] B. Renshaw, J. Neurophysiol. 9, 190 (1946).

*Original Russian pagination. See C.B. translation.

THE PARABIOTIC NATURE OF THE REACTION OF THE NERVOUS SYSTEM DURING THE ACTION OF GAS GANGRENE TOXIN

S. V. Magaeva

Laboratory of Infectious Pathology (Head—Corresponding Member AMN SSSR Prof. A. Ya. Alymov, Scientific Director—Dr. Med. Sci. O. Ya. Ostryi) of the Institute of Normal and Pathological Physiology (Director—Active Member AMN SSSR V. N. Chernigovskii), AMN SSSR, Moscow

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

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An analysis of the clinical picture of gas gangrene gives grounds for the assumption that the bacterial toxins have a paralyzing action on the centers of the medulla oblongata [1-3, 9, 10].

Several investigations have shown [4-7, and others] that the infectious toxicosis in gas gangrene is brought about by two fundamental nervous mechanisms: reflex and "automatic." The nervous origin of the toxic manifestations is shown particularly clearly by the extreme reactivity of the central nervous system to the bacterial toxin of the causative agent of gas gangrene and by the possibility of the prevention of the development of these toxic manifestations by surgical division of the reflex arc at any link, by lowering the reactivity of the nervous system by pharmacological methods or, finally, by protecting the central nervous system with specific antitoxic serum. The authors cited above largely investigated the reflex mechanism of the pathological process: The summational principle of its mechanism was revealed and the importance of the various divisions of the reflex arc in the development of the disease was demonstrated.

The results obtained dealt a severe blow to the widely held local hypothesis of the toxic manifestations affecting the organs in gas gangrene [8, 11, 12, and others].

It accordingly became necessary to investigate the nature and the special features of the reaction of the nervous system to the action of the toxins in order to develop further the study of the pathogenesis of gas gangrene, and this formed the purpose of our present research.

METHOD AND RESULTS

The work was undertaken on white rats and frogs. We used the toxin of one of the anaerobes of the main "group of four" (A. V. Mel'nikov)—vibrion septique (Series 405 and 406 from the N. F. Gamaleya Institute of Epidemiology and Microbiology).

The reaction of the nervous system to the action of the toxin was investigated initially in experiments on a peripheral nerve.

Liquid toxin (4 mg/0.1 ml) was applied to the sciatic nerve by means of a cotton wool swab or by injection of the toxin (0.4 mg in 0.02 ml) beneath the membrane of the nerve trunk. An insulating polyethylene film was placed beneath the nerve. The changes in the functional properties of the nerve were investigated in acute experiments by the method of parabiosis, as adopted by N. E. Vvedenskii's school. Recordings were made of the reaction of the gastrocnemius muscle to stimulation of the sciatic nerve by an induction current, using electrodes situated in the area affected by the toxin or proximally to it.

The action of the toxin of vibrion septique on the sciatic nerve was shown by disturbances of the afferent and efferent innervation of the limb, lasting several days. The muscles of the affected limb were atonic; the limb was not included in the act of locomotion. The animal did not react to nociceptive stimulation of the skin in the region of the 4th-5th digits.

The experiments showed that the changes undergone by the excitation of a peripheral nerve after the application of toxin to it have the characteristic three phases of the process of parabiosis. In the first phase, the excitation of the nerve fell by 7-10% in the course of 5-10 minutes; in the second phase it rose by 10-15% in the course of 10-15 minutes, and in the third phase it again fell, this time by 45-90% in the course of 40-60 minutes (Fig. 1, a).

The phase of the secondary fall in excitation was most characteristic of a nerve affected by toxin, and developed with the typical stages of parabiosis, of which

the most constant and prolonged was the stage of equilibration. The reaction of "inhibition immediately after excitation" was often apparent. The first two phases of parabiosis were inconstant and were not shown after injection of the toxin beneath the membrane of the nerve trunk.

Investigation of the functional properties of the nerve, affected by toxin, at various intervals after injection of toxin beneath its membrane showed the presence of lowered excitation and of stages of parabiosis for 8-10 days, with gradual restoration of the excitation and conduction of the nerve. Analysis of the changes in the physiological lability of the nerve in the third phase of its reaction to the action of toxin was undertaken by the usual method of electrotonic change in the functions. A constant current was applied by means of Dubois-Raymond nonpolarizing electrodes. The active pole of the current was situated in the altered area of the nerve, and the indifferent pole in the tibial muscles of the leg. A layer of insulating material was placed beneath the nerve.

The creation of an anode in the affected area of the nerve in 11 of the 19 experiments briefly restored the conduction of the nerve (at a current strength of 0.4-0.8 ma). The cathode intensified the parabiotic process (Fig. 1).

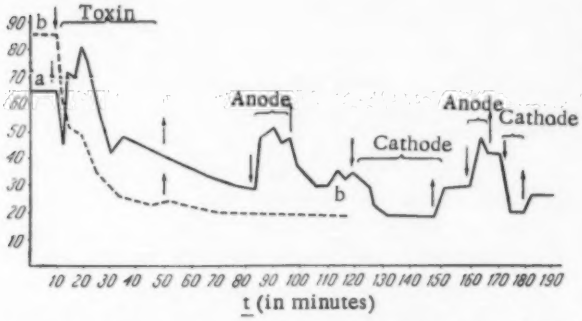

Fig. 1. Change in the excitation of a nerve when affected by toxin. a—Changes in the excitation of the sciatic nerve, and the effect of the electrode on the alteration of the properties of the nerve; b—changes in the excitation of the peripheral end of the undivided nerve. Along the vertical axis, the threshold of stimulation of the nerve (in divisions of the induction scale).

The character of the electrotonic changes in the functions of the nerve affected by toxin and the presence of stages of parabiosis were evidence of lowered lability in the most characteristic (the third) phase of the reaction of the nerve to the toxin of vibrion septique.

The experiments showed that the subordinating effect of the central nervous system and the anelectrotonic influence on the course of the parabiosis and in this particular form of nerve lesion were in the same direction. Parabiosis of the divided nerve developed much more quickly after the application of toxin than did the reaction of the subordinated nerve, and took place without the manifestation of the first two phases of the process (Fig. 1, b).

The reaction of the myoneural synapse to the action of the toxin was investigated in experiments in which toxin was injected into the proximal portions of the gastrocnemius muscle and the reactions of the muscle to indirect and direct stimulation were recorded. The experiments showed a rapid disturbance of the conductivity of the myoneural synapse under the influence of the toxin, while the excitation of the muscle was itself preserved for some time.

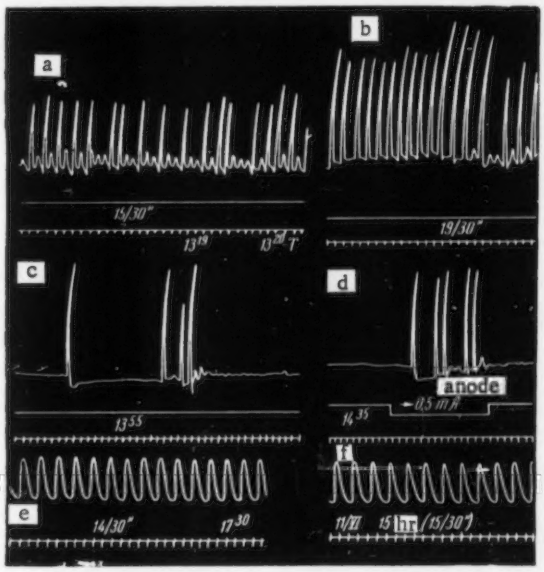

Fig. 2. Changes in the respiratory movements and the mechanocardiogram of a frog after the application of toxin to the medulla oblongata. a—Normal respiratory movements; b—changes in respiration 3 minutes after application of the toxin to the medulla oblongata; c—changes in respiration after 30 minutes; d—restoration of respiration after the creation of anelectro-tonus in the region of the medulla oblogata at the 32nd minute of cessation of pulmonary respiration; e—mechanocardiogram of the frog 3½ hours after cessation of pulmonary respiration; f—mechanocardiogram on the 3rd day after cessation of pulmonary respiration.

The reaction of the centers of the spinal cord and medulla to the action of the test toxin was studied in experiments on frogs.

The action of toxin on the centers of the spinal cord was brought about by applying the toxin to the outer surface of the cord by means of a cotton wool swab (2 mg in 0.1 ml). Recordings were made of the reflex reaction of the gastrocnemius muscle after stimulation of the central end of the tibial nerve with an induction current.

The experiments showed that, after application of toxin to the spinal cord, a parabiotic reaction developed, with features as described above.

The fact that the frog can cutaneously compensate for pulmonary respiration enabled us to investigate advanced stages of changes in the central regulation of respiration and the activity of the heart.

These investigations were carried out by applying the toxin (0.4 g/0.1 ml) to the exposed medulla oblongata or by injection of the toxin into the forebrain. Recordings were made of the respiratory movements of the buccal membrane.

After the action of the toxin on the brain, in several cases the rate of pulmonary respiration was raised and its strength was increased. In all the experiments periodic respiration developed 40-60 minutes after administration of the toxin, after which complete cessation of the respiratory movements ensued (Fig. 2).

The immobility of the animal, the atony of the skeletal musculature, and the absence of a motor defensive reaction to nociceptive stimulation were obvious evidence of irradiation of inhibition from the brain centers to the underlying divisions of the central nervous system.

After the complete cessation of respiratory movements, the activity of the heart, according to the data from the mechanogram, underwent no essential changes and its rate was gradually slowed on the 3rd-5th day. The frog could survive from 3 to 7 days, right until death, in a state of profound inhibition of the centers of pulmonary respiration. Analogous changes in respiration took place after injection of the toxin (2.8 mg in 0.1 ml) into the dorsal lymph sac of the frog, which indicated that some degree of similarity existed between the reaction of the animal to the application of toxin to the medulla oblongata and the effect produced by toxin circulating in the body. The rhythmic activity of the respiratory center, when inhibited by toxin, could be restored for a short time by stimulation of the skin of the animal in the first 5-7 minutes after cessation of respiration.

We also investigated the possibility of the electrotonic restoration of respiration. For this purpose the swab with the toxin was removed after respiration had ceased for 1-3 minutes; the active pole of the current was placed on the surface of the affected area of the brain and the indifferent pole on the skin in the region of the sacrum. The creation of anelectrotonus in the affected area of the medulla oblongata led to restoration of respiratory movements during the period of application of a current of 0.25-0.5 ma in 29 cases (6 of 14 experiments) (see Fig. 2).

The injection of strychnine into the lymph sac (0.2 ml of a 0.01% solution) 40 minutes before injection of the toxin into the brain prevented the cessation of pulmonary respiration and enabled survival in 17 of 20 animals whereas all the control animals died. We considered that the prophylactic action of strychnine which we observed was due to the labilization of the respiratory center. All the experiments described were accompanied by suitable controls of the effect of the nonspecific component of the bacterial toxin on the functional state of the nervous system.

The control experiments showed that the toxin, when destroyed by boiling or neutralized by specific antitoxic serum, did not cause the reaction described above. This was evidence that the parabiosis-producing properties were confined to the specific component of the vibrion septique toxin.

The parabiotic reaction of the central and peripheral nerve structures which we observed during the action of the toxin of one of the agents responsible for gas gangrene evidently reflected the "automatic" component of the toxic manifestations of this disease which we were studying. The distinctive features of the action of the vibrion septique toxin on the medulla was evidence of the decisive importance of the parabiotic process of the respiratory center in the pathogenesis of the toxicosis.

It may be postulated that, in ordinary circumstances, the development and outcome of the pathological process are to a large extent determined by the complex and dynamic relationships between the reflex and "automatic" components of the toxicosis, appearing in the form of a single parabiotic process of the nervous system.

SUMMARY

This work deals with the effect exercised by the toxin of one of the causative agents of gas gangrene on the functional condition of the nervous system (experiments on rats and frogs). The author demonstrated the parabiotic nature of reactions of the respiratory center, the spinal cord centers, the peripheral nerve, and of the myoneural synapse in their alteration with the toxin of vibrion septique.

The peculiarity of this reaction lies in the phase of decreased lability and excitability and the prolonged equalizing stage of parabiosis. As shown by experiments on frogs, the parabiotic process of the respiratory center is of especial pathogenetic significance. The function of the central nervous and peripheral nervous formations, disturbed during the process of alteration by the toxin, may be restored for a short period by creating an anelectrotonus in the area of alteration. Prophylactic administration of strychnine may prevent the development of a parabiotic process of the respiratory center in the frog.

LITERATURE CITED

[1] D. A. Arapov, Anaerobic Wound Infection (Moscow, 1950) [In Russian].

[2] V. Ya. Braitsev and S. P. Zaeva, Novyi Khirurg. Arkh. 30, 4, 472 (1934).

[3] A. N. L'vov, Gas Gangrene (Moscow, 1946) [In Russian].

[4] O. Ya. Ostryi and A. N. Aliev, Doklady Akad. Nauk SSSR 106, 1, 157 (1956).

[5] O. Ya. Ostryi and A. N. Aliev, Doklady Akad. Nauk SSSR 115, 2, 421 (1957).*

*Original Russian pagination. See C.B. translation.

[6] O. Ya. Ostryi, Z. I.Sobieva, and A. N. Aliev, Current Problems of Nervism in Physiology and Pathology (Moscow, 1958), p. 724 [In Russian].

[7] Z. I. Sobieva, Current Problems of Nervism in Physiology and Pathology (Moscow, 1958), p. 691 [In Russian].

[8] W. Frei, Ergebn. allg. Path. and Path. Anat. 31, 1 (1936).

[9] C. H. Kellaway, E. R. Trethewie, and A. W. Turner, Austral. J. Exper. Biol. Med. 18, 225 (1940).

[10] F. Klose, Munchen. med. Wschr. 64, 1541 (1917).

[11] M. B. Robertson, A System of Bacteriology in Relation to Medicine (London, 1929) 3, p. 225.

[12] W. Straub, Munchen. med. Wschr. 66, 89 (1919).

THE CORRELATION BETWEEN THE ACTIVE CONCENTRATION OF LABELED AMINO ACIDS BY THE CELLS, AND THEIR INCLUSION IN THE PROTEINS OF TUMORS AND NORMAL TISSUES OF EXPERIMENTAL ANIMALS IN VITRO

K. A. Perevoshchikova

Laboratory of Biochemistry (Head—Prof. I. B. Zbarskii, Scientific Director— Corresponding Member of the AMN SSSR A. I. Savitskii) of the P. A. Herzen State Oncological Institute (Director—Prof. A. N. Novikov), Moscow

(Presented by Active Member of the AMN SSSR A. E. Braunshtein)

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Several authors have demonstrated that the concentration of free amino acids is higher in the tissues [16] and erythrocytes [1] than in the blood plasma. It was more recently found that the entry of amino acids into cells is due to a process of active concentration, as opposed to a gradient of concentrations with the consumption of energy [7,12,14].

The inclusion of labeled amino acids in the proteins of tumor sections in vitro is known to be considerably more intense than in the case of normal tissue sections [17]. In homogenates and other acellular preparations made from tumors, this process is no more intense than in analogous preparations made from normal tissue [6].

Since the first stage in the utilizationof amino acids for protein synthesis in their accumulation by the cell, one can propose that the rate of protein synthesis and the growth of tissues in the organism should depend on the ability of the cells to concentrate amino acids. Because tumor cells (ascitic cells of Ehrlich's cancer) possess a heightened ability to accumulate amino acids [11], I. B. Zbarskii has expressed the hypothesis that the greater inclusion of labeled amino acids in tumor cell proteins in vitro can be explained by the increased ability of these cells to accumulate amino acids [2].

In this work, in order to prove this hypothesis, we investigated in vitro the correlation between the concentration of radioactive amino acids by ascitic cells, and by sections of experimental tumors and normal tissues, and the inclusion of such amino acids in the cell proteins.

METHOD

We studied the concentration of glycine-1-C^{14} methionine-S^{35} lysine-1-C^{14} and tyrosine-1-C^{14} by the cells of Ehrlich's ascitic mouse cancer, sarcoma M-1, and rat's ascitic hepatoma.*

The normal tissue used for purposes of comparison was that of rat liver. Two-hundred mg sections were incubated in Robinson's medium with glucose [15] in Warburg's flasks at 37.8° for 30 minutes in an oxygen atmosphere. The radioactive amino acid was added to the flasks in an amount of 30,000-70,000 impulses per 2 ml of the incubation medium.

After incubation, the sections were briefly dried with a filter paper, weighed, ground up, and precipitated with trichloracetic acid in a final concentration of 2.5%. The free amino acids were extracted by thrice extracting the sections with 2.5% trichloracetic acid. The deproteinized incubation medium and the trichloracetic filtrates were neutralized and reduced to a specific volume, from which the sample for radioactivity determination was taken— in the case of glycine, quantitative determination, according to Alexander's method [4], was also done. After the extraction of the free proteins, the proteins of the sections were processed by the standard method [3]. The radioactivity of theproteins was calculated on a "B" type apparatus per every 10 mg protein. The results were expressed as the number of impulses per minute for a specific volume of the incubation medium and for an equal volume of the original tissue.

RESULTS

Table 1 gives the ratio of the radioactivity of the protein-free filtrates of cells or sections to the radioactivity of the incubation medium. As the data in the table indicate, the cells of the experimental tumors concentrated amino acids much more actively than did the cells of the normal liver. This ability of the cells depended on the amino acid employed as well as on the type of experimental tissue. Under these conditions,

* This strain was kindly made available to us by the Laboratory of Strains of the AMN SSSR Institute of Experimental Cancer Pathology and Therapy.

TABLE 1. Concentration of Glycine-C^{14}, Methionine-S^{35}, Lysine-C^{14} and Tyrosine-C^{14} in vitro by Tumor and Liver Cells from Rats and Mice. Ratio of number of impulses per minute in a protein-free filtrate of cells or sections to the number of impulses per minute in an equivalent volume of the incubation medium (30-minute incubation at 37.8° in Robinson's medium. Average values from 7-15 experiments)

Tumor	Intracellular glycine-C^{14} / Glycine-C^{14} in incubation medium	Intracellular methionine-S^{35} / Methionine-S^{35} in incubation medium	Intracellular lysine-C^{14} / Lysine-C^{14} in incubation medium	Intracellular tyrosine-C^{14} / Tyrosine-C^{14} incubation medium
Rat's sarcoma M-1	4.1 ± 0.30	3.0	2.0 ± 0.08	2.3 ± 0.20
Ehrlich's ascitic mouse sarcoma	8.5 ± 1.70	–	3.1	2.9
Rat's ascitic sarcoma	3.3	–	2.1	–
Rat liver	1.3 ± 0.24	1.4	0.8 ± 0.14	1.3 ± 0.10
Mouse liver	1.3	–	–	–

TABLE 2. Correlation Between the Active Concentration of Amino Acids and Their Inclusion in the Proteins of Rat's Sarcoma M-1 and Rat Liver

Amino acids used	Number of experiments	Radioactivity of free amino acids in tumor cells / Radioactivity of free amino acids in liver cells	Number of experiments	Radioactivity of tumor proteins / Radioactivity of liver proteins
Glycine-C^{14}	14	2.9 ± 0.26	15	2.8 ± 0.29
Methionine-S^{35}	5	2.0	5	1.7
Tyrosine-C^{14}	9	1.8 ± 0.26	10	6.3 ± 0.75
Lysine-C^{14}	9	2.0 ± 0.17	9	9.0 ± 1.5

glycine was most actively concentrated by the cells of Ehrlich's ascitic mouse cancer (an average of 8 1/2 times).

In some of the experiments, the concentration of glycine inside the ascitic cancer cells was 20 times higher than the content of this amino acid in the incubation medium. The variations in the concentrating activity of the cells were probably due to disregarded biological factors. Comparing the individual experiments gave us reason to suppose that the stage of the tumor's development if of essential importance.

The concentration of lysine and tyrosine by ascitic cells of Ehrlich's cancer is less intense than that of glycine, but less variable. In almost all the experiments, the concentration of tyrosine and lysine within the cancerous cells was 2-3 times higher than the concentration of these substances in the medium.

The effect observed with the sections of solid tumors was similar. For example, the ratio of the glycine concentration in the cells of sarcoma M-1 to that in the incubation medium was 4.1. In individual experiments, this ratio reached 8-11. The use of Alexander's method for quantitative determination of glycine produced results which coincided with the data obtained from the determination of radioactivity; in the succeeding experiments, therefore, we used only the isotope method. The concentration of lysine-C^{14} and tyrosine-C^{14} in the cells of sarcoma M-1 was twice that in the incubation medium. The concentration of the amino acids in the cells of rat's ascitic hepatoma was 2-3 times higher than in the external environment. Therefore, the tumor cells in the experimental animals were extremely active in regard to the concentration of amino acids. Moreover, the concentration of the experimental labeled amino acids in the rat and mouse liver cells was only 1.3 times higher than their concentration in the medium. The concentration of lysine-C^{14} in the liver cells was even lower than in the incubation medium.

A comparison of the concentrating activity of the cells with the inclusion of amino acids in the cells' proteins (Table 2) will show that the rate of glycine-C^{14} and mehtionine-S^{35} inclusion in the proteins of sarcoma M-1 and rat liver was practically proportional to the ability of these cells to concentrate these amino acids. In fact the ratio of the radioactivity of the free amino acids of the tumor to that of the liver was almost identical to the ratio between the radioactivity figure for the proteins of these cells. These ratios were 2.9 and 2.8 respectively for glycine and 2.0 and 1.7 for methionine (see Table 2). The individual experiments as well as the average values showed this conformity. In one of the experiments, for example, the ratio of the active concentration of amino acids by the cells of a sarcoma M-1 to

their concentration by the liver cells equalled 4.4, while the radioactivity of the tumor proteins was 4 1/2 times greater than that of the liver proteins.

The concentration of tyrosine-C^{14} was an average of 1.8 times higher in the tumor cells than in the liver cells. The rate of tyrosine inclusion in the tumor proteins was 6 times higher than the inclusion of this amino acid in the liver proteins. In this case, as in the case of lysine (for which these figures were, respectively, 2 and 9), with the relatively high concentration of these amino acids by the tumor cells, their inclusion in the protein as compared with the liver cells was considerably higher than their concentratability. In other words, under the experimental conditions described, the ability of the cells to actively concentrate tyrosine and lysine was not proportional to the rate of the inclusion of these amino acids in the tissue proteins.

According to the literature date [see 7,9,10,11], tumor and embryonic tissues have an extremely pronounced ability to actively concentrate amino acids.

Since the first stage in protein synthesis is the utilization of amino acids by the cells, the ability of tumor cells to accumulate amino acids extremely actively could be the main reason for the intensified protein synthesis typical of malignant neoplasms. Until recently, however, tumor cells, particularly ascitic cells of Ehrlich's cancer, have only been used as a convient object on which to study the mechanism of active concentration.

Our experiments showed that cells of sections of sarcoma M-1 and rat's ascitic hepatoma, as well as ascitic tumor cells, possess a greater ability in experiments in vitro than liver cells for the active concentration of amino acids. These data are in accord with the research of Miller and Burke [see 5], which demonstrated that the level of amino acids in the liver cells is higher after the development of a true hepatoma than during the precancer period.

The high concentration of amino acids by tumors is evidently connected with the rate of protein synthesis. In fact, cells of tissues in which the processes of protein synthesis are extremely intense are especially active in the concentration of amino acids. In this respect, the fact that there is a higher level of amino acids in embryonic tissues than in the maternal tissues is particularly interesting. For example, the concentration of glycine in the skeletal muscles of a guinea pig fetus [see 7] is three times higher than that in the muscles of the maternal organism.

Neoplastic cells concentrate amino acids especially actively. In our experiments, the glycine uptake by the ascitic cells of Ehrlich's cancer was an average of 8.5 times (in some experiments, 15-20 times) the uptake by the incubation medium, while the uptake by the cells of sarcoma M-1 was up to 7-8 times (an average of 4 times) the uptake by the medium. The accumulation of the other amino acids by these cells (lysine-C^{14}, tyrosine-C^{14} methionine-S^{35}) was somewhat less intense. The extraordinarily intense inclusion of amino acids in the proteins of tumor cells is in keeping with their high concentration activity. In the case of glycine-C^{14} and methionine-S^{35}, we observed a direct proportionality between these processes. Loftfield and Eigner [13] discovered a similar relation in their research on the synthesis of ferritin in rat's liver carried out in vivo with the aid of leucine-C^{14}.

The increase in the radioactivity of the whole liver protein in this work was proportional to the increase in the activity of the intracellular amino acids. The lack of a proportion between the ratios showing the rate of amino acid inclusion in the protein and the active concentration of these acids by tumor and normal cells observed in the experiments with tyrosine and lysine still does not exclude the possibility that there is a more complex relationship between these processes.

Therefore, cells of sarcoma M-1, rat's ascitic hepatoma, and Ehrlich's ascitic mouse cancer actively concentrate glycine-C^{14}, methionine-S^{35}, tyrosine-C^{14} and lysine-C^{14}. The capacity of the tumor cells for the active concentration of amino acids was 2-8 times greater than that of the liver cells.

The ratio of the inclusion rate of labeled glycine and methionine in the proteins of slices of sarcoma M-1 to the inclusion rate ot these amino acids in the liver proteins equalled the ratio of the uptake of these amino acids by the cells of the tissues named. In the case of labeled tyrosine and lysine, the relative intensity of inclusion in the tumor protein was considerably higher than the relative ability of the tumor cells to concentrate these amino acids.

We regard the results obtained as confirmation of the hypothesis that the increased inclusion of amino acids in tumor proteins in vitro is largely due to the increased ability of tumor cells to concentrate amino acids.

SUMMARY

It is shown that the cells of sarcoma M-1, ascitic hepatoma of rats, and Ehrlich's ascitic cancer of mice actively concentrate glycine-C^{14}, methionine-S^{35}, tyrosine-C^{14}, and lysine-C^{14}. The uptake of amino acids by tumor cells is 2-8 times higher than the uptake by the liver cells.

The ratio of the incorporation rate of labeled glycine and methionine in the proteins of sarcoma M-1 slices to the incorporation rate in the liver proteins in equal to the ratio of the uptake of the corresponding free amino acids by the cells of the mentioned tissues. In the case of labeled tyrosine and lysine, the relative rate of incorporation in the tumor protein is considerably higher than the relative ability of the tumor cells to concentrate these amino acids. The results obtained are regarded as confirmation of the hypothesis that the increased inclusion of amino acids in vitro in tumor proteins is largely due to the increased ability of tumor cells to concentrate amino acids.

LITERATURE CITED

[1] I. B. Zbarskii and N. N. Demin, The Role of Erythrocytes in Protein Metabolism [In Russina] (Moscow, 1949).

[2] I. B. Zbarskii, "Tumor proteins and their metabolism", in: Essential Problems of Modern Biochemistry (Vol. 1, The Biochemistry of Proteins) [In Russian]. (1959) p. 233.

[3] I. B. Zbarskii and K. A. Perevoshchikova, Biokhimiya 22, 1-2, 295 (1957).*

[4] B. Alexander, G. Landwehr, and A. M. Seligman, J. Biol. Chem. 160, 51 (1945).

[5] W. T. Burke and L. L. Miller, Feder. Proc. 14, 187 (1955).

[6] P. N. Campbell and O. Greengard, Biochem. 66, 47 (1957).

[7] H. N. Christensen and J. A. Streicher, J. Biol. Chem. 175, 95 (1948).

[8] H. N. Christensen, J. A. Streicher and R. L. Eblinger, J. Biol. Chem. 172, 515 (1948).

[9] H. N. Christensen, T. R. Riggs, H. Fischer et al., J. Biol. Chem. 198, 1 (1952).

[10] H. N. Christensen, B. Hess, and T. R. Riggs, Cancer Pres. 14, 124 (1954).

[11] H. N. Christensen in: Symposium on Amino Acid Metabolism (Baltimore, 1955), p. 63.

[12] H. N. Christensen, T. R. Riggs, A. J. Aspen et al., Ann. New York, Acad. Sci. 63,983 (1956).

[13] R. B. Loftfield, E. A. Eigner, J. Biol. Chem. 231, 925 (1958).

[14] T. R. Riggs and L. M. Walker, J. Biol. Chem. 233, 132 (1958).

[15] J. R. Robinson, Biochem. J. 45, 68 (1949).

[16] D. D. Van Slyke and G. M. Meyer, J. Biol. Chem. 16, 197 (1913-1914).

[17] P. C. Zamechnik, I. D. Frantz, R. B. Loftfield et al., J. Biol. Chem. 175, 299 (1948).

THE EFFECT OF ISOHEMOTRANSFUSION ON THE METABOLISM OF ADRENERGIC SUBSTANCES IN THE BRAIN AND EFFECTOR ORGANS

R. M. Glants

Physiological Laboratory (Dir.—R. M. Glants, Candidate of Medical Sciences) of the Ukrainian Institute for Scientific Research in Blood Transfusion (Dir.—Chief Scientific Co-worker, Yu. M. Orlenko)

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

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In 1957, we established that isohemotransfusion and primary transfusion of therapeutic serum (LSB) prepared by N. G. Belen'kii's method cause a pronounced and prolonged increase in the norepinephrine content of rabbit' hearts. Further development of this research led us to study of isohemotransfusion on the metabolism of adrenergic substances in the brain, striated muscle (triceps brachii), and the spleen.

METHOD

Rabbits were given a transfusion of freshly preserved (according to recipe No. 7 of the Order of Lenin Central Institute of Hematology and Blood Transfusion) rabbit's blood in a dose of 3-5 ml per kg of animal weight. Thirty minutes, 24, and 72 hours after the isohemotransfusion, the animals were decapitated, and the epinephrine-like substances in the brain, striated muscle (triceps brachii), and spleen were investigated by V. O. Osinskaya's method [11]. This method makes it possible to determine norepinephrine, epinephrine, compounds with the properties of oxidation products of epinephrine-like substances, and non-adrenal fluorescence. The experimental and control animals were killed on the same day. Changes in the metabolism of adrenergic substances were estimated by comparing the data obtained from the experimental and control animals in the course of one experimental day and also by comparison with the average level of the corresponding adrenergic substance in the control rabbits. Investigations were performed on 58 rabbits (30 experimental, 14 control, and 14 donors). The data were processed by the method of variable statistics (D—0.95-0.99).

RESULTS

The investigations conducted showed that isohemotransfusion is attended by a prolonged and pronounced increase in the norepinephrine content of the brain (Fig. 1, a, b). Even 72 hours after the transfusion, the norepinephrine content of the brain was greater then in the control rabbits.

Thirty minutes and twenty-four hours after the isohemotransfusion, the content of epinephrine-like substances was increased. After 72 hours, the content of these substances was the same as in the control rabbits in one animal and less than in the control in seven animals. Only in two cases was the content of epinephrine-like substances greater than in the control animals (Fig. 1, c, d). The fact that we hardly ever found epinephrine present in any of the experimental tissues is in accord with the literary data [14].

The changes in the metabolism of the striated muscle after isohemotransfusion (Fig. 2) followed the same course as those observed in the brain, although the changes observed in the content of epinephrine-like substances were more pronounced.

After isohemotransfusion, we also observed an increase in the content of norepinephrine and epinephrine-like substances [oxidation products (Fig. 3)] in the spleen. The metabolism of adrenergic substances in the brain, striated muscle, and spleen showed similar changes after primary transfusions of the heterogenic blood substitutes BK_8 [protein-blood substitute (protein hydrolyzate)] and LSB [13].

Norepinephrine is a total peripheral vasoconstrictor which exerts its action at its place of formation [15]. The increase in the content of adrenergic substances in the brain, heart, and main blood depots (striated muscle and spleen) is an extremely important part of the mechanism of the hemodynamic effect produced by various kinds of hemotherapy. It is no exaggeration to propose that pathology of the vascular tonus is pathology of the arterial pressure regulators. From the physiological viewpoint, therefore, one cannot concur with the opinion widely held that the mechanism of hemotherapy's hemodynamic effect is only the mechanical filling of the vascular system with blood colloids or plasma.

The data cited, together with the material we published earlier, show that different kinds of hemotherapy alter the functional condition of different sections of the corticovisceral reflex arc: the angioreceptors, the

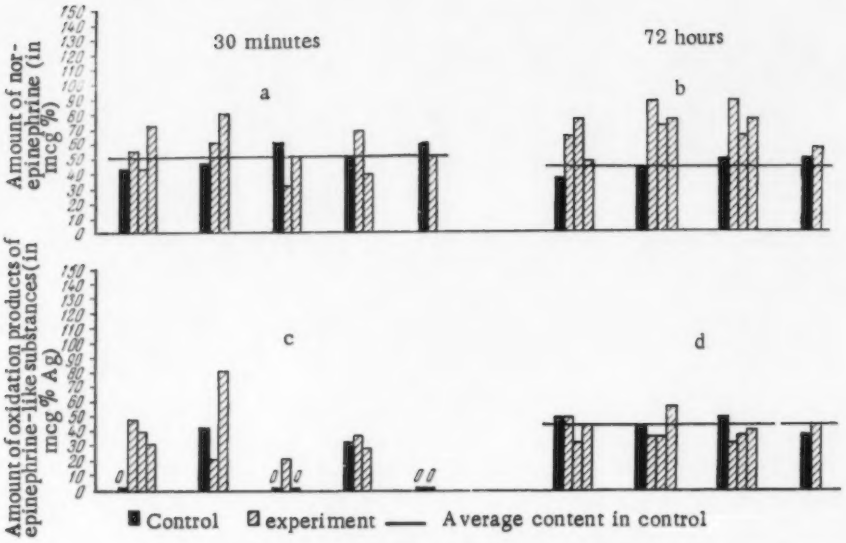

Fig. 1. Change in the amount of norepinephrine and epinephrine-like substances in the brain. Key: a, c—30 minutes; b, d—72 hours after isohemotransfusion.

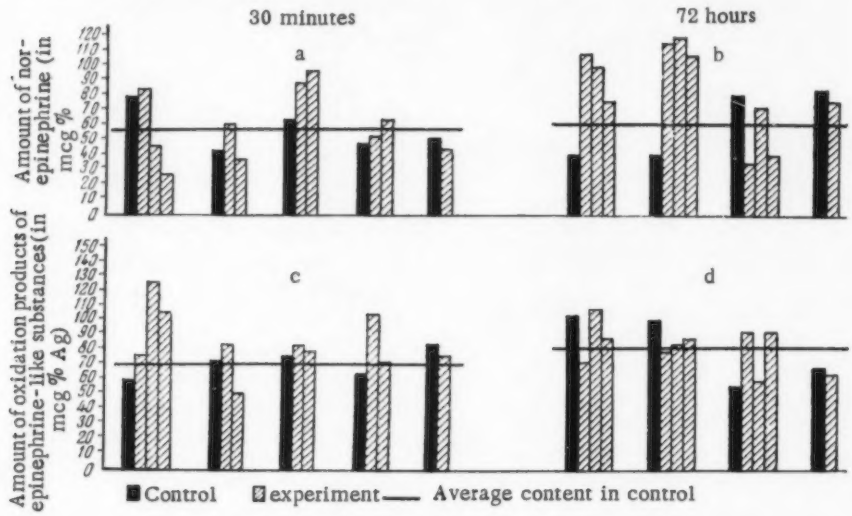

Fig. 2. Change in the amount of norepinephrine and epinephrine-like substances in the striated muscle. Symbols the same as in Fig. 1.

cerebral cortex, sections of the autonomic nervous system, and the endocrine glands. One can consider it established [1-10, 12, 13] that isohemotransfusion and transfusion of heterogenic blood substitutes are attended by a decrease in the epinephrine content of the adrenal glands, an increase in the content of epinephrine-like substances in the blood, an increase in the content of norepinephrine and oxidation products of epinephrine-like substances in the brain and effector organs (heart, striated muscle and spleen), change in the function of the anterior lobe of the hypophysis, increased excretion of corticoids into the blood by the cortical part of the adrenal glands, and intensification of the function of the posterior lobe of the hypophysis, attended by an increased amount of vasopressin in the blood.

Therefore, the different types of hemotherapy have a very substantial effect on the function of the main regulators of vascular tonus, and this, one must suppose, is the foundation of the mechanism of their hemodynamic effect.

Our data indicate that one must approach the action mechanism of transfused blood or blood substitutes from the standpoint that the transfused blood interacts with the organism, so that the organism plays a definite part in the effect of transfused blood. This, of course, also applies to heterogenic blood substitutes.

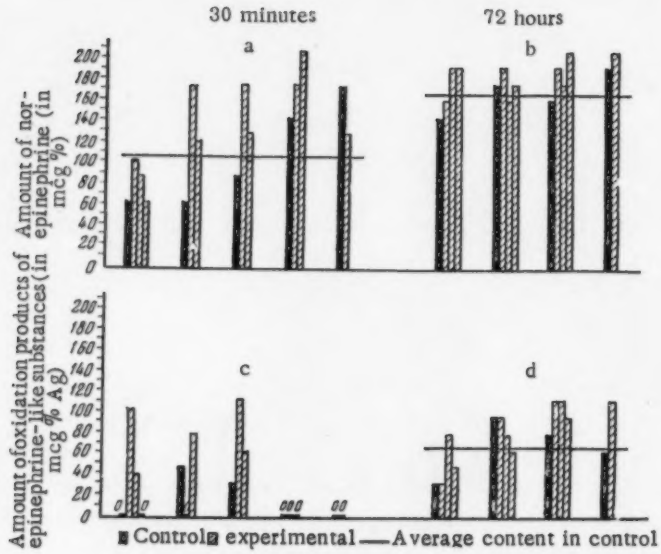

Fig. 3. Change in the amount of norepinephrine and epinephrine-like substances in the spleen. Symbols the same as in Fig. 1.

SUMMARY

The author studied the effect of isohemotransfusion on the content of norepinphrine, epinephrine, and compounds possessing the properties of the oxidation products of epinephrine-like substances in the brain and the main blood depots (the striated muscle triceps brachii, and the spleen).

As established, isohemotransfusion is attended by an increase in the amount of norepinephrine and oxidation products in all the tissues investigated. The main feature in the hemodynamic action of various types of hemotherapy is the effect on the functional condition of the systems controlling the arterial blood pressure, i.e., the nervous and the endocrine.

LITERATURE CITED

[1] R. M. Glants, Vrachebnoe Delo 11, 983 (1953).

[2] R. M. Glants, Voprosy Fiziol. 5, 52 (1953).

[3] R. M. Glants, Transactions of the 8th Conference of Surgeons of the Ukrainian SSR [In Russian] (Kiev, 1955), p. 376, 506.

[4] R. M. Glants, Vrachebnoe Delo 2, 111 (1955).

[5] R. M. Glants, Problemy Gematol. i Pereliv. Krovi 3, 42 (1956).

[6] R. M. Glants, Vrachebnoe Delo 9, 921 (1956).

[7] R. M. Glants, Byull. Eksptl. Biol. i Med. 12, 45 (1958).*

[8] R. M. Glants, Byull. Eksptl. Biol. i Med. 4, 46 (1959).*

[9] R. M. Glants, Abstracts of the Proceedings of the Jubilee Session of the Tbilisi Institute for Scientific Research in Blood Transfusion [In Russian], (Tbilisi, 1958) p. 4.

[10] R. M. Glants, in: Collected Scientific Transactions of the Erevan Institute of Hematology and Blood Transfusion 7-8 [In Russian] (Erevan, 1959) p. 146.

[11] V. O. Osinskaya, Biokhimiya 3, 537 (1957).*

[12] N. I. Panchenko, Vrachebnoe Delo 3, 203-206 (1954).

[13] M. G. Pasternak, in: Problems of Blood Transfusion 5 [In Russian] (Khar'kov, 1958) p. 88.

[14] A. M. Utevskii, in: Problems of the Biochemistry of the Nervous System (Kiev, 1957), p. 131 [In Russian].

[15] W. Raab, in: The Progress of Cardiology [Russian translation] (Moscow, 1959) p. 67.

*Original Russian pagination. See C. B. translation.

THE EFFECT OF PAIN STIMULATION ON THE PHAGOCYTIC POWER OF THE LEUKOCYTES IN HORSES

A. F. Rusinov

Department of General and Special Surgery (Head—Honored Scientist of the Ukr. SSR, the late Prof. V. A. German) of the Khar'kov Veterinary Institute

(Presented by Active Member AMN SSSR V. V. Parin)

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It is clear from data in the literature that the phagocytic function of the leukocytes is regulated by the nervous system and is increased by pain stimulation [4, 5, 7, 9, 11, etc.]. Although the leukocytes are feed blood cells and have no anatomical innervation, their phagocytic activity is regulated by mediators secreted into the blood stream by the nerve endings of the vegetative nervous system. Sympathin causes increased phagocytosis and "vagus-stoff" causes its depression.

METHOD

The changes in the phagocytic function of the leukocytes during the healing of wounds were studied in 18 horses under various conditions: without anesthesia, with ordinary lumbar paravertebral block, and with prolonged lumbar paravertebral block.

The phagocytic index was determined by the following method: Into two sterile agglutination tubes, each containing 0.05 ml of a 6% solution of sodium citrate, was poured 0.2 ml of whole blood obtained from the jugular vein. After agitation of the contents of the tubes, to one of them was added 0.25 ml of a standard suspension of streptococci, and to the other, the same volume of a standard suspension of Staphylococcus aureus. The suspensions consisted of washings of a 24-hr culture of these microorganisms, and contained 2×10^9 bacterial cells/ml. The blood was mixed with the suspension of the microorganisms and then allowed to stand in the incubator at 38° for 30 minutes. It was essential that a fresh bacterial suspension be prepared before the reaction by washing a 24-hr culture of streptococci or staphylococci with physiological saline. The bacterial suspension was prepared from permanent strains of streptococcus and staphylococcus, which we obtained from the Department of Microbiology of the Khar'kov Veterinary Institute. The ingested bacterial cells were counted in 200 leukocytes.

During the experiments the animals were kept on the same diet and in the same conditions. The phagocytic index was investigated before the infliction of incised or crushing wounds of the skin and muscles in the iliac region, and subsequently after 15-30 min, 1-2-3-4-6-8 hr and 1-2-3-4-5-6-7-10-12-15-20 days of the injury (only individual examples are given in the tables). We investigated the phagocytic index so frequently because, in our experiments, it was an indirect sign of the degree of stimulation of the nervous system as the result of pain impulses from the region of the wound.

RESULTS

In all the experiments the phagocytic index rose sharply immediately after infliction of the wounds, being 1.5-2 times larger after only 15 minutes. After ordinary anesthesia, the phagocytic index remained essentially unchanged at the end of 4-6 hours by comparison with the first and second hours, and it then began to increase gradually again (Table 1).

In the horses in which anesthesia was not used, the index continued to rise during the next 2-4-6-8 hours. Usually in these cases the phagocytic index rose until 24 hours. Occasionally the increase was less pronounced. After 24 hours a fall in the phagocytic index was observed, with a return to its initial value at the end of 4-5 days. In animals in which prolonged anesthesia with a 5% solution of procaine in horse fat (or in peach oil) was used, the phagocytic index rose only very slightly to 6-8 hours by comparison with the first and second hours; it then gradually fell and reached its initial level at 24 hours or, occasionally, at 48 hours (see Table 1).

The phagocytic index changed especially demonstratively when an additional stimulus was used—the instillation of concentrated sulfuric acid onto the wound surface (Table 2).

After infliction of the wounds, as in the previous experiments, the phagocytic index increased sharply—by 1.5-2 times in comparison with the initial values; then, after instillation of sulfuric acid on the wound surface without anesthesia, it continued to rise sharply for the first two days.

With ordinary anesthesia, the increase in the phagocytic index was delayed immediately after anesthesia and for the next 2-4 hours, and it then continued to rise until 24-48 hours. In both cases the phagocytic index be-

TABLE 1. Results of Determinations of the Phagocytic Index in Experiments on Horses

Name of horse and record no.	Conditions of wound healing	Phagocytic index[1]																
		before wounding	after															
			15-30 min	1 hr	2 hr	4 hr	6 hr	8 hr	1 day	2 days	3 days	4 days	5 days	6 days	7 days	10 days	15 days	20 days
The mare Astra, No. 1	Without anesthesia	15	25	29	36	38	38	40	55	25	20	16	14	15	15	14	13	14
The mare Galka, No. 4	With ordinary anesthesia. . .	7	12	12	14	14	15	19	24	11	10	10	7	8	7	8	9	7
The mare Kukla, No. 13	With prolonges anesthesia . .	11	25	28	28	28	27	28	14	10	10	12	10	10	10	12	11	10

[1]The number of ingested microorganisms was counted in 200 leukocytes

TABLE 2. Results of Determinations of the Phagocytic Index in Experiments on Horses Using An Additional Stimulus – Sulfuric Acid

Name of horse and record no.	Conditions of wound healing	Phagocytic index[1]																
		before wounding	after															
			15-30 min	1 hr	2 hr	4 hr	6 hr	8 hr	1 day	2 days	3 days	4 days	5 days	6 days	7 days	10 days	15 days	20 days
The mare Lastochka, No. 24	Without anesthesia + sulfuric acid	25	52	56	60	70	79	90	98	98	65	34	28	25	23	22	23	24
The stallion Linok, No. 21	Sulfuric acid + ordinary anesthesia.	23	42	44	60	60	68	90	94	99	67	35	29	26	27	29	25	25
The stallion Myach, No. 21	Sulfuric acid + prolonged anesthesia	25	47	50	55	57	66	70	40	22	22	23	23	24	23	24	24	23

[1]The number of ingested microorganisms was counted in 200 leukocytes

gan to fall at the end of the second day after infliction of the wounds, and returned to its initial values at the end of the fifth or sixth day. It remained perceptibly unchanged during the next 10-15 days.

With prolonged anesthesia, the phagocytic index rose only in the first 6-8 hours after infliction of the wounds and instillation of sulfuric acid, after which it began to fall and reached its initial values at the end of 24-48 hours. In the next 15-20 days it was almost unchanged.

What is the explanation of the changes in the phagocytic index after the infliction of wounds without anesthesia and with the subsequent use of ordinary and prolonged anesthesia?

Pain is one of the factors causing excitation of the sympathetic nervous system. It is well known that the sympathetic nervous system is distinguished by its very high excitability [8]. It is pertinent to quote A. N. Bekulev's statement that "a wound is an extensive receptor surface which is the source of pathological impulses to the cerebral cortex, causing its exhaustion" [1].

Among the many pathological impulses, those due to pain occupy a prominent place. From the data in the literature and our personal investigations, we consider that, immediately after the infliction of wounds, especially with the subsequent instillation of sulfuric acid onto the wound surface, the intensity of pain in the region of the wound increases. The pain stimulation, acting reflexively through the nervous system, causes a series of defensive reactions, including an increase in the phagocytic power of the leukocytes. In our experiments the increase in this power was observed for 48 hours. It may be suggested that, during this period, the strongest pathological impulses (with pain dominant among them) pass from the wound to the central nervous system, and that these caused the well-marked increase in the phagocytic index. As these pathological impulses, including those due to pain, weakened, the phagocytic power of the leukocytes diminished.

Bearing in mind the changes in the phagocytic index, it must be considered that the pathological impulses (including those due to pain) were strongest in the first phase of healing (5-7 days after infliction of the wounds), especially when sulfuric acid was subsequently instilled onto the wound surfaces. They later became weaker, so that at the end of 5-7 days the phagocytic index returned to its initial value; after 5-7 days, i.e., in the second and third phases of the healing process, it remained substantially unchanged.

The ordinary or prolonged lumbar paravertebral block which we used prevented the transmission of pain stimulation to the central nervous system. Under local anesthesia the peripheral receptors in the injured tissues are blocked, so that the reflex influence of the "products" of pain is decreased. Removal of the pain and other impulses, passing from the region of the wound to the central nervous system, delays the increase in the phagocytic activity of the leukocytes.

With ordinary anesthesia the phagocytic index did not change appreciably for two hours, and then continued to rise. It must be assumed that this was due to the fact that the peripheral receptors in the injured tissues were blocked for two hours, as a result of which the humoral "products" of pain were not formed, and their reflex influence was thereby prevented. With prolonged local anesthesia, the block of the peripheral receptors continued longer; during this time no humoral "products" of pain were evidently formed, in consequence of which the phagocytic index not only did not increase, but returned comparatively quickly to its initial level. In this connection it has to be pointed out that most authors [2, 3, 6, 10, etc.] consider the opsono-phagocytic reaction to be very sensitive, reflecting the condition of the body.

It will be apparent from the findings described that, in animals in which no anesthesia was used, the phagocytic index rose sharply immediately after infliction of the wounds and during the next two days, after which it gradually fell and reached its initial values at the end of 5-7 days, i.e., when the acute inflammatory processes had subsided and granulation tissue had begun to develop in the wound. In the second and third phases of healing of the wound the phagocytic index remained substantially unchanged.

Ordinary and prolonged anesthesia delayed the increase in the phagocytic index. We may remark that during this time the number of phagocytes in the wound itself increased perceptibly, from our observations made on impressions from the wound surface, in those animals in which the phagocytic index was studied.

The method of taking impressions enabled us to judge objectively the intensity of the measures taken by the body to resist infection, and the course of cleansing of the wounds from microorganisms and necrotic tissues, both with and without anesthesia. For the sake of brevity we will describe the comparative findings for the neutrophil group alone.

During the systematic analysis of the wound impressions in all the animals, we observed the following. In horses in which especially prolonged anesthesia was used during healing of the wounds, the cytogram during the first 4-6 days showed a large number of neutrophils in various stages of phagocytic activity. The number of dead leukocytes in a state of disintegration was relatively small. Free-lying microorganisms were almost nonexistent or were found as isolated specimens. The microorganisms were completely ingested by the neutrophils and showed a varying intensity of staining, indicating the active bacteriolysis (digestion) of the microorganisms, a sign of the high protective power of the animal. At the same period of wound healing without anesthesia, the impressions showed a less marked phagocytosis, many neutrophils without signs of phagocytosis and many dead leukocytes in various stages of disintegration. Around the neutrophils were seen large collections of microorganisms that had not undergone phagocytosis.

SUMMARY

As shown by investigations, the phagocytic index may to a certain extent objectively reflect the removal of pain and other pathological impulses coming from the wound area into the central nervous system. Proceeding from the literature data and his own observations, the author also considers that the phagocytic activity of leukocytes is an objective indication of the condition of the organism, the course of the wound process, and the efficacy of the therapeutic measures.

LITERATURE CITED

[1] A. N. Bakulev, Zhur. Vysshei Nerv. Deyatel. 3, 319 (1951).

[2] G. D. Belonovskii, Transactions of a Conference on Bacteriology, Epidemiology, and Leprosy [In Russian] (St. Petersburg, 1911) p. 3.

[3] V. K. Vysokovich, Transactions of a Conference on Bacteriology, Epidemiology, and Leprosy [In Russian] (St. Petersburg, 1911) p. 69.

[4] A. G. Gel'dyeva, Abstracts of Proceedings of a Conference of Pathophysiologists of Central Asia and Kazakhstan [In Russian] (Stalinabad, 1955) p. 19.

[5] I. N. Golovkova, Byull. Eksptl. Biol. i Med. 24, 4, 268 (1947).

[6] P. T. Kozyr', Parallel Clinical and Immunological Trends in Patients with Aseptic and Suppurative Processes, Candidate's Dissertation [In Russian] (Khar'kov, 1949).

[7] K. N. Muksinova, The Phagocytic Activity of the Leukocytes of the Blood of Animals in Various Functional States of the Cerebral Cortex. Author's abstract of dissertation [In Russian] (Ufa, 1955).

[8] S. P. Protopopov, The Pathogenesis and Treatment of Indolent Wounds [In Russian] (Moscow, 1950).

[9] N. V. Puchkov, Current Problems of General Pathology and Medicine [In Russian] (Moscow, 1950) p. 123.

[10] T. I. Stepanyuk, Microcide as a Factor in the Variation of Streptococci and Staphylococci in Vitro and in Vivo, Candidate's dissertation [In Russian] (Khar'kov, 1951).

[11] P. P. Firsova, Vrachebnoe Delo 4, 325 (1953).

THE EFFECT OF THE SECRETION OF THE CUTANEOUS GLANDS OF THE TOAD ON CERTAIN IMMUNOLOGICAL PROPERTIES OF ANIMALS

V. I. Zakharov, N. V. Kolpikov and B. M. Kurtser

Department of General Biology (Head—Prof. V. I. Zakharov) and the Department of Pathological Physiology (Head—Dr. Med. Sci. E. P. Kuchinskii) of the Kishinev Medical Institute (Dir.—Honored Scientist Prof. N. T. Starostenko)

(Presented by Active Member AMN SSSR V. V. Parin)

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Biologically active substances are widely distributed in living organisms. Items of special interest are the animal poisons, which possess high activity. One such product of biological synthesis is the secretion of the cutaneous glands of the toad (SCGT).

The therapeutic and toxic action of the secretion of the cutaneous glands of various toads has been known for a long time. According to E. N. Pavlovskii [10], the Chinese many centuries ago prepared from the poisonous cutaneous glands of the common toad (Bufo vulgaris) the therapeutic preparation "Sen-So," which they used as a cardiac drug. The literature [1, 8] contains toxicological and pharmacological information on the poison of the toad. On the basis of N. P. Kravkov's researches, I. P. Pavlov [9] put forward the idea that SCGT possesses a mixed action on the body.

The action of this secretion has been studied [11, 12] on isolated organs and on intact animals.

Systematic investigations of the character of the action of SCGT on animals were made by V. I. Zakharov [2-6]. He studied the effect of SCGT on animals of different species, or of the same species but at different stages of individual development. He also studied the effect of SCGT on warm-blooded animals, in normal conditions and in certain functional disorders reproduced experimentally.

Several authors have shown [7] that SCGT is an effective stimulator of leuko- and thrombopoiesis in white rats after exposure of these animals to x-rays.

These investigations provided the basis for the study of the action of SCGT on certain immunological processes in animals.

Among the protective adaptations developing in man and animals during the process of evolution, an important place is occupied by antibodies and the phagocytic properties of the fixed and mobile cells of the body.

In the present investigation the action of SCGT was studied on antibody formation and on the phagocytic activity of the leukocytes of the blood.

METHOD

Experiments were carried out on white rats and male rabbits. The animals were immunized by the subcutaneous injection of a killed typhoid vaccine. SCGT was injected into the animals subcutaneously—into rats in a dose of 1 ml of a concentration of 1:1000; into rabbits in a dose of 1 ml/kg body weight of a concentration of 1:2000. In all, three series of experiments were performed on 12 rabbits and 46 rats. In the first series of experiments the action of SCGT was studied on the changes in the agglutinin titer in rabbits; in the second series its action on the formation of agglutinins on rats were studied; finally, in the third series, the action of SCGT was studied on the phagocytic activity of the leukocytes in rats.

The agglutinin titer was determined by the agglutination reaction, the intensity of which was read by means of an agglutinoscope. The phagocytic activity of the leukocytes was determined against a 24-hr culture of Staphylococcus aureus on agar.

RESULTS

The first series of experiments was carried out on 12 rabbits, of which six were experimental and six control (the latter were immunized but did not receive SCGT). After the initial agglutinin titer had been determined, the animals of the experimental group were injected with SCGT in a dilution of 1:2000 and a dose of 1 ml/kg body weight. Immunization began on the fifth day with a suspension of a typhoid culture (containing 1×10^9 bacterial cells/ml) in a dose of 0.3-0.5 ml. The animals were vaccinated five times at intervals of five days. The rabbits received SCGT four times during the period of immunization, so that the antigen was injected during the time of action of the secretion.

The control group of rabbits received injections of physiological saline (0.85% NaCl) in a dose of 1 ml/kg body weight.

TABLE 1. Agglutinin Titer of the Serum of Rabbits Vaccinated with Typhoid Vaccine and Receiving SCGT

Animal no.	Before vaccination	After vaccination and injection of SCGT			
		after 9 days	after 16 days	after 23 days	after 28 days
2	1:25	1:1 600	1:800	1:400	1:400
3	—	1:1 600	1:400	1:200	1:50
4	—	1:800	1:1 600	1:800	1:100
5	1:25	1:3 200	1:400	1:400	1:200
6	1:25	1:6 400	1:12 800	1:3 200	1:800
7	—	1:1 600	1:3 200	1:800	1:100

TABLE 2. Agglutinin Titer of the Serum of Rabbits Vaccinated with Typhoid Vaccine

Animal no.	Before vaccination	After vaccination			
		after 7 days	after 15 days	after 25 days	after 30 days
1	1:25	1:400	1:800	1:400	1:200
2	1:25	1:1 600	1:800	1:400	1:100
3	—	1:100	1:400	1:400	1:200
5	1:25	1:200	1:50	1:25	—
6	1:25	1:3 200	1:800	1:400	1:200
7	—	1:400	1:200	1:200	1:50

TABLE 3. Agglutinin Titer of the Serum of the Control and Experimental Rats

No. of control animal	On the tenth day after vaccination	No. of experimental animals	On the 11th day after vaccination and injection of SCGT
1	Negative	1	1:3 200
2	»	2	1:3 200
3	»	3	1:1 600
4	1:400	4	1:3 200
5	1:200	5	1:6 400
6	Negative	6	1:400
7	1:400	7	Discarded from the experiment
8	Negative	8	1:800
9	Discarded from the experiment	9	1:6 400
10	1:800	10	1:1 600

Blood for estimation of the agglutinin titer was taken from the experimental animals 9, 16, 23, and 28 days after vaccination, and from the control animals 7, 15, 25, and 30 days after vaccination.

The results obtained in the first series of experiments are shown in Tables 1 and 2.

It will be seen from the figures in Table 1 that in three cases the agglutinin titer reached 1:1600-1:3200 on the ninth day; on the 16th day the titer was observed to fall. In the other three cases the titer on the ninth day was 1:800, 1:6400, and 1:1600; on the 16th day it had risen to 1:1600, 1:12,800, and 1:3200, and after 23

TABLE 4. Phagocytic Activity of the Leukocytes in Rats after Subcutaneous Injection of SCGT

Animal no.	Phagocytic activity of leukocytes (in %)						
	Before injection of SCGT	After injection of SCGT					
		after 2 days	after 6 days	after 10 days	after 15 days	after 25 days	after 35 days
1	13	25	24	22	21	—	—
2	16	20	21	18	20	—	—
3	13	19	34	27	26	21	20
4	15	16	30	21	23	25	20
5	21	13	36	25	26	20	—
7	18	14	23	22	27	24	17
8	17	10	22	22	24	15	—
9	17	12	18	16	15	17	18
10	14	19	24	17	16	18	17
11	15	27	31	21	19	20	19
12	11	31	24	24	12	13	14
13	12	20	27	18	11	12	—
14	16	35	32	19	14	18	17
15	18	15	10	13	13	11	10
16	15	18	23	17	16	15	14
17	18	30	31	28	17	20	19

Note. A minus sign (–) means that the phagocytic activity was not determined in these cases.

TABLE 5. Phagocytic Activity of the Leukocytes in the Rats of the Control Group

Animal no.	Phagocytic activity of leukocytes (in %)					
	before injection of physiological saline	after injection of physiological saline				
		after 2 days	after 6 days	after 10 days	after 15 days	after 25 days
1	13	14	12	13	14	12
2	17	16	15	14	17	—
3	12	11	12	10	11	13
4	6	7	8	7	6	—
5	12	13	10	11	12	12
6	10	11	10	9	11	10
7	11	14	10	12	13	—
8	15	12	12	11	13	14
9	10	11	12	9	10	—
10	8	9	7	10	9	10

Note. A minus sign (–) means that the phagocytic activity was not determined in these cases.

days a fall in the agglutinin titer was observed in every case.

In the control group (Table 2) the highest agglutinin titer was observed in four cases on the seventh day after vaccination (1:200, 1:3200, 1:400, and 1:1600); on the 15th day in these cases a fall in the titer was observed. In two cases on the seventh day the titer was 1:400 and 1:100; on the 15th day it reached 1:800 and 1:400. On the 25th day the titer was lowered in every case.

It thus follows from analysis of the results obtained that SCGT considerably increases the antibody-forming (agglutinins) power of animals.

The second series of experiments was conducted on 20 rats (weight 150-200 g), kept on an ordinary diet; 10 of these were used as controls and were only im-

munized, whereas the experimental animals received, in addition, three injections of SCGT (1 ml, 1:1000) during the period of immunization.

The animals were immunized with a suspension of a typhoid culture (1×10^9 bacterial cells/ml), receiving six injections, each of 0.1 ml, at intervals of 3 days.

On the 10th and 11th days after vaccination and injection of SCGT, blood was taken for investigation of the agglutinin titer.

The results obtained in this series of experiments are shown in Table 3. These results show that SCGT caused a sharp increase in the agglutinin titer in the white rats.

In the third series of experiments the effect of SCGT was studied on the phagocytic activity of the leukocytes in rats. The investigation was carried out on 26 rats, ten of which were controls. After the determination of the initial phagocytic activity of the leukocytes, the experimental animals received three injections of SCGT (1 ml, 1:1000) at intervals of 2-3 days. The control animals received subcutaneous injections of 1 ml of physiological saline at the same times.

At intervals of 2, 6, 10, 15, 25, and 35 days, blood was taken from the animals for estimation of the phagocytic activity of the leukocytes. As material for phagocytosis a suspension of Staphylococcus albus in physiological saline was used.

The results of this series of experiments are shown in Tables 4 and 5.

The results of the last series of experiments show that in 14 of the 16 experimental animals there was a considerable increase in the phagocytic activity of the leukocytes. The maximum increase of activity of the leukocytes was observed two and six days after injection of the SCGT. In three cases, a considerable fall in the phagocytic activity was observed two days after the injection of SCGT, but after six days the activity of the leukocytes exceeded the original values.

One animal (No. 15) showed a lowering of the phagocytic activity throughout the whole experiment, and in another case (No, 9) the activity remained within the limits of physiological variation. In the majority of animals the figures regained their initial values after 25-35 days.

In the control group of animals the phagocytic activity of the leukocytes showed no significant difference from the initial values throughout the entire experiment.

In this series of experiments the SCGT thus had a marked stimulating action on the phagocytic activity of the leukocytes of the blood.

SUMMARY

This work deals with the effect of the secretion of the cutaneous glands of toads on the dynamics of the agglutinin titer in rabbits and rats, as well as on the phagocytic activity of blood leukocytes in rats. The data obtained demonstrate that this substance increases the ability of the animal to form agglutinins, and stimulates the phagocytic activity ofthe blood leukocytes in rats.

LITERATURE CITED

[1] A. V. Zakusov, Russk. Vrach 15 (1904).

[2] V. I. Zakharov, The Poison of Toads (An Experimental Investigation). Doctorate dissertation (Alma-Ata, 1952) [In Russian].

[3] V. I.Zakharov, Byull. Eksptl. Biol. i Med. 32, 8, 135 (1951).

[4] V. I. Zakharov and I. P. Krichevskaya, in: Problems of Experimental Biology and Medicine 2, 197 (Moscow, 1952) [In Russian].

[5] V. I.Zakharov, Byull. Eksptl. Biol. i Med. 35, 1, 65 (1953).

[6] V. I. Zakharov, Nauchnye Izvest. Kazakhsk. Med. Inst. Alma-Ata 12, 105 (1955).

[7] N.V. Kolpikov, V. I. Zakharov, B. M. Kurtser, et al., Zdravookhranenie 3, 42 (1958).

[8] N. P. Kravkov, Russk. Vrach 21, 761 (1904).

[9] I. P. Pavlov, Complete Collected Works 5, (Moscow-Leningrad, 1949) p. 358 [In Russian].

[10] E. N. Pavlovskii, Venomous Animals of the USSR (Moscow-Leningrad, 1931) pp. 4, 60, 66, 98 [In Russian].

[11] F. F. Talyzin and A.A. Pchelkina, Doklady Akad. Nauk SSSR 66, 2, 301 (1949).

[12] A. I. Shumeino, Trudy Samarkandskogo Med. Inst. 4, 50 (1939).

THE ELECTROPHYSIOLOGICAL ANALYSIS OF THE ACTION OF ANTIGENS ON ANGIOCEPTORS

A. N. Gordienko, V. I. Kiseleva, R. B. Tsynkalovskii, B. A. Saakov, Ya. I. Azhipa, A. V. Let'en, A. I. Egorov, L. N. Ochelenko, I. M. Bondarev, and L. I. Zhigalina

Department of Pathophysiology (Head—Prof. A. N. Gordienko) of the Rostov Medical Institute

(Presented by Academician A. D. Speranskii)

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In our previous research we found that the injection of an antigen into the isolated carotid sinus leads to the production of specific antibodies, in spite of the removal of the depot of antigen after five minutes [3, 4, 5]. These findings were confirmed by the investigations of E. A. Tatarinov [9], V. A. Tychinin [10] and B. G. Avetikyan [1].

At the same time, G. V. Shumakova [11], and Z. M. Prusakova [8] point out that the production of antibodies in this form of immunization takes place only in the presence of resorption of the antigen. A. D. Ado [2] considers that antigens have the power to cause stimulation of the receptors of the carotid sinus only when immunological reactivity is modified, and in the absence of such a modification they do not possess this power.

In this worker's opinion, an antigen cannot reflexively cause the production of antibodies, for it does not have the power to cause stimulation of the receptors on first contact.

We have shown that the intradermal injection of vaccines of different microorganisms causes stimulation of the receptors of the skin, as a result of which a stream of afferent impulses passes along the sensory fibers [6]. Pathogenic microorganisms of the enteric group stimulate the receptors of the carotid sinus and cause changes in the action potentials in the carotid sinus nerve [7].

In the present communication we describe the changes in the action potentials in the carotid sinus nerve during the action of staphylococcal, whooping cough, and Escherichia coli antigens on the receptors of the carotid sinus.

METHOD

Experiments were carried out on dogs. The carotid sinus nerve was isolated under hexobarbital anesthesia and was surrounded by a ligature. All the major vascular branches, except the common carotid artery, were ligated 1-1.5 cm from the bifurcation. The carotid sinus nerve was placed in a rubber tube in which electrodes were inserted and affixed in order to assure reliable contact. The action potentials were recorded on a loop oscillograph. Type 2-kub-2 amplifiers and the animals were placed in a screened room, providing reliable protection from electrostatic and electromagnetic effects.

Antigen, in a dose of 0.3-0.5 ml, was injected through the common carotid artery. The vaccines used were prepared by the Rostov Institute of Vaccines and Sera and contained 4×10^9 bacterial cells/ml.

Four series of experiments were performed, with 10-12 animals in each.

RESULTS

The initial background of the action potentials in the carotid sinus nerve showed characteristic discharges of rapid waves, which followed different intervals of time (0.5-3.25 sec) in individual animals. The amplitude of these waves reached 5-20 μv. In each discharge, from 20 to 40 waves were counted. In the intervals between the discharges the bioelectrical activity of the carotid sinus nerve was weak.

In the first series (12 experiments) the action of staphylococcal vaccine was studied. Injection of the vaccine in all the experiments caused changes in the bioelectrical activity of the carotid sinus nerve, but of varying degree. As a rule (nine experiments) the amplitude of the waves of potential forming a discharge was increased 1.5-2 times and reached 8-40 μv. The number of waves in the discharge increased two- or three-fold, to as much as 90 waves per second. The amplitude of the interdischarge potentials was increased, sometimes to reach the magnitude of the original background discharge waves. In the majority of cases the reaction developed after 0.5-1.5 min and occasionally it was immediately after injection of the vaccine. Normalization of the action potentials was observed after 3-10 min. In the remaining three experiments, after injection of the

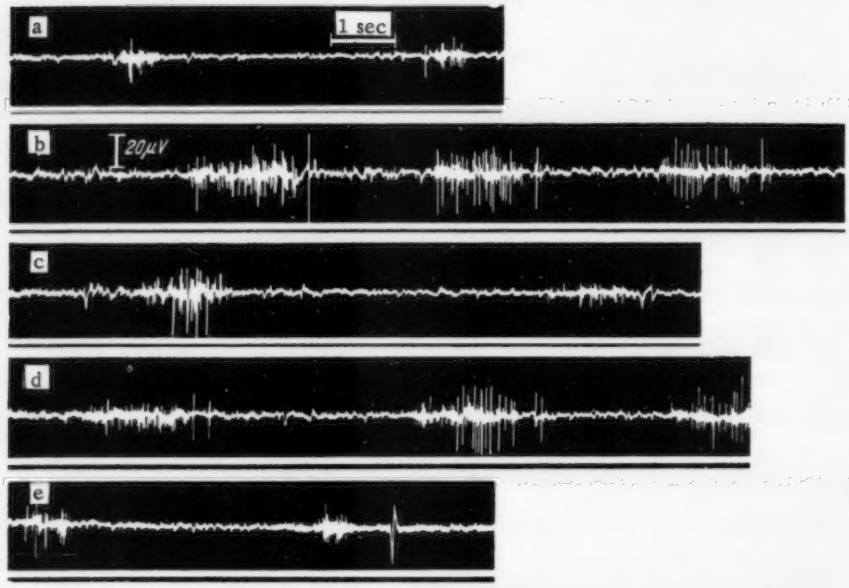

Fig. 1. Electroneurogram of the carotid sinus nerve after the action of staphylococcal antigen. a—Initial background; b—at the moment of injection of the antigen; c, d, e—1, 2, and 5 minutes after injection of the antigen.

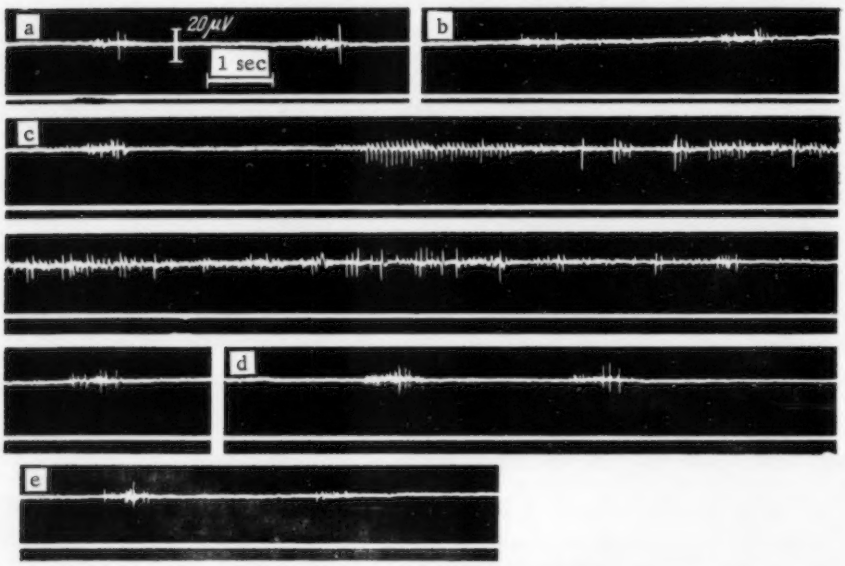

Fig. 2. Electroneurogram of the carotid sinus nerve after the action of whooping cough vaccine. a—Initial background; b—at the moment of injection of antigen; c, d, e—1, 3, and 5 minutes after injection of antigen.

antigen the bioelectrical activity in the interdischarge period was unchanged, but the amplitude of the discharge waves increased to 50% of the initial magnitude.

The change in the character of the flow of impulses after injection of staphylococcal antigen into the carotid sinus can be seen from the photocopies of the electroneurograms given below (Fig. 1).

In the second series (12 experiments) the bioelectrical activity of the carotid sinus nerve was studied during the action of whooping cough antigen on the recep-

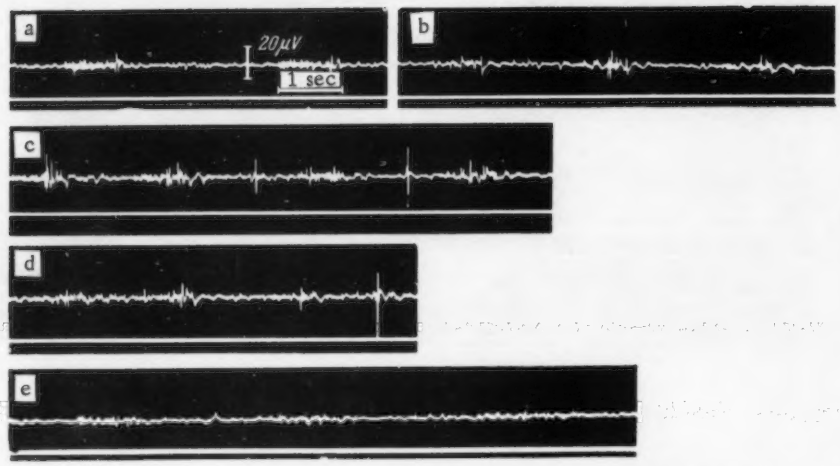

Fig. 3. Electroneurogram of the carotid sinus nerve after the action of E. coli vaccine. a—Initial background; b, c, d, e—1, 3, 5, and 10 minutes after injection of antigen.

tors of the carotid sinus. Before injection of the antigen the ordinary flow of impulses in the carotid sinus nerve, as described above, was recorded.

After injection of the vaccine the character of the action potentials of the carotid sinus nerve was changed: Monophasic or, more often, biphasic waves with sharp apices were recorded, the amplitude of which reached 8-25 μv. In some cases groups of waves appeared, with a frequency of 10 to 18 per second. The amplitude of these waves reached 7-14 μv. The reaction lasted for two or three minutes and the action potentials then returned to their initial state (Fig. 2).

The electrophysiological characteristics of the reaction of the carotid sinus nerve in this case differed from those of the reaction observed in response to the action of staphylococci on the receptors of the carotid sinus (see Figs. 1 and 2).

In the third series (10 experiments) the bioelectrical activity of the carotid sinus nerve was studied during the action of E. coli antigen. The injection of this antigen into the carotid sinus caused little change in the action potentials of the carotid sinus nerve. In some cases the character of the groups of discharges was changed, and occasionally solitary monophasic and biphasic waves appeared (Fig. 3).

The study of the character of the changes in the potentials in the carotid sinus nerve after injection of physiological saline into the carotid sinus (fourth series of experiments) enabled the effect of changes of pressure due to the injection of that particular volume of fluid to be estimated. When the common carotid artery was not ligated, the possibility of mechanical stimulation from the injection of 0.3-0.5 ml of fluid was unlikely. Nevertheless, we considered it necessary to perform a series of control experiments in which sterile physiological saline was injected. The injection of physiological saline did not alter the action potentials of the nerve, either at the moment of injection or at different periods thereafter. The character of the oscillograms throughout the whole time after the injection of physiological saline showed no significant difference from that of the initial background (Fig. 4).

Electrophysiological analysis of staphylococcal and whooping cough antigens and of E. coli on the vascular receptors of the carotid zone shows very convincingly that these agents possess the ability to stimulate the receptors of the carotid sinus. The stimulation is transformed into a process of excitation and this excitation reveals itself by a particular type of neurogram. E. coli antigen possesses this property to a lesser degree.

The changes in the action potentials in the carotid sinus nerve cannot be explained by mechanical stimulation of the receptors, since injection of the same volume of physiological saline as a rule caused no changes in the action potentials in the carotid sinus nerve.

It should be mentioned that, during comparison of the electroneurograms recorded after the injection of staphylococcal and whooping cough antigens and, to a lesser degree, E. coli antigen, certain differences are found, as shown by the characteristic changes in the discharges of waves of potential, the configuration of the discharges themselves, and the bioelectrical activity in the intervals between the discharges.

From the results obtained it may be concluded that the quality of the excitation in the receptors is different during the action of different antigens, and that this may, possibly, determine the special features of the reaction of the body to various pathogenic stimuli.

SUMMARY

Staphylococcal antigen produces irritation of the carotid sinus receptors; this is transformed into excitation,

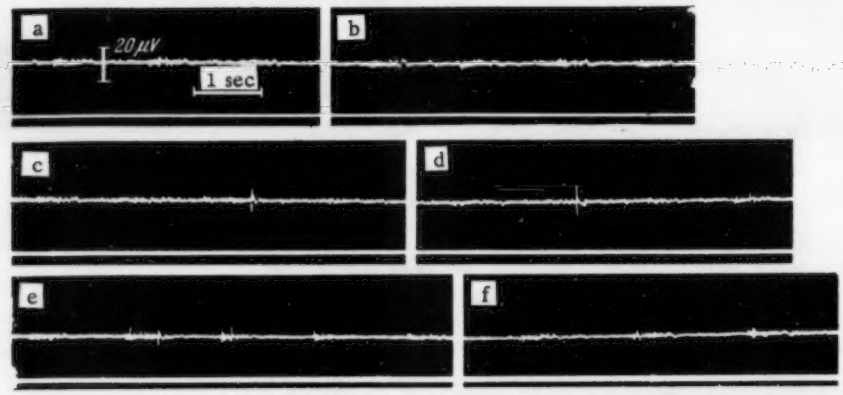

Fig. 4. Electroneurogram of the carotid sinus nerve after injection of physiological saline (control). a—Initial background; b—at the moment of injection of the solution; c, d, e, f—1, 2, 5, and 10 minutes after injection of the solution.

which gives rise to fluctuating bioelectric potentials of monophasic and biphasic character. The discharge period is increased, while in the intervals between the discharges the initial fluctuation of the potentials becomes intensified. Pertussis antigen also irritates the carotid sinus receptors and thus produces in the carotid sinus nerve a flow of impulses changing the normal oscillogram of this nerve. E.coli has a weak ability to irritate the carotid sinus receptors and changes only insignificantly the fluctuation rate of the carotid sinus nerve bioelectric potentials. The variations in the action currents of the carotid sinus nerve, originating from the action of the antigens on the carotid sinus receptors, are independent of any mechanical irritation. Administration of the same amount of physiological saline has no effect on the oscillogram of the sinus nerve.

LITERATURE CITED

[1] B. G. Avetikyan, Zhur. Mikrobiol. Epidemiol. i Immunobiol. 5, 52 (1956).

[2] A. D. Ado, Antigens as Extraordinary Stimuli of the Nervous System (Moscow, 1952) [In Russian].

[3] A. N. Gordienko, R. B. Tsynkalovskii, and B. A. Saakov, Byull. Eksptl. Biol. i Med. 38, 9, 45 (1954).

[4] A. N. Gordienko, R. B. Tsynkalovskii, and B. A. Saakov, Byull. Eksptl. Biol. i Med. 38, 12, 45 (1954).

[5] A. N. Gordienko, R. B. Tsynkalovskii, and B. A. Saakov, Med. Zhur. Ukrain. 24, 6, 8 (1954).

[6] A. N. Gordienko, V. I. Kiseleva, B. A. Saakov, et al., Byull. Eksptl. Biol. i Med. 1 (supplement), 147 (1957).•

[7] A. N. Gordienko, Abstracts of Proceedings of the Second All-Union Conference of Pathophysiologists (Kiev, 1956) p. 82 [In Russian].

[8] Z. M. Prusakova, in: Problems of Age and Reactivity in Infectious and Immunological Processes (Leningrad, 1955) p. 236 [In Russian].

[9] E. A. Tatarinov, Abstracts of Proceedings of the First All-Union Conference of Pathophysiologists (Moscow, 1950) p. 64 [In Russian].

[10] V. A. Tychinin, The Importance of the Receptors of the Carotid Sinus and Spleen in Immunogenesis and Anaphylaxis, Author's abstract of candidate dissertation (Kiev, 1951) [In Russian].

[11] G. V. Shumakova, in: Problems of Infectious Pathology and Immunology (Moscow, 1954), p. 29 [In Russian].

•Original Russian pagination. See C.B. translation.

CHANGES IN THE MORPHOLOGY OF CONNECTIVE TISSUE EXPLANTATES IN RATS TREATED WITH METHYLCHOLANTHRENE AND EXTRACT FROM A HUMAN LUNG TUMOR

M. T. Mogila and Ya. V. Dobrynin

Laboratory of Tissue Culture of the Division of Etiology and Pathogenesis of Tumors (Head—Active Member AMN SSSR A. D. Timofeevskii) of the Institute of Experimental Pathology and Therapy of Cancer (Dir.—Corresponding Member AMN SSSR Prof. N. N. Blokhin), AMN SSSR, Moscow

(Presented by Active Member AMN SSSR A. D. Timofeevskii)

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That malignant change may occur in tissues outside the body must be regarded as proven. Evidence of this is given by the work of Earle and his co-workers [20], A. D. Timofeevskii and his co-workers [14–18], and others. The question of the direct causes of malignant change in explantates, however, remains open. In A. D. Timofeevskii's opinion, malignant change of tissues regularly takes place in explantates only after the combined action of carcinogenic substances and tumor-producing viruses, which could be demonstrated experimentally [2-6, 14-18]. It was shown, moreover, that malignant change may arise after the action of an extract from certain tumors, the virus nature of which was not proven [6]. A similar effect was observed after the action of an extract of a human lung carcinoma on a connective tissue culture in rats [10]. The observations mentioned above, along with others, suggest that in certain tumors there is a virus-like principle which causes malignant change in the tissues. On this basis the problem of the virus nature of neoplasms, including tumors in man, could be considered.

According to certain reports [19, 21], in a number of human malignant tumors, globular corpuscles are found with considerable regularity, and revealing definite virus-like properties [1, 7, 8, 9, 11, 12, 13]. The study of the malignant effect of this agent on connective tissue explants is of definite interest.

In our experiments we investigated the effect of the combined action of methylcholanthrene and an extract of a human lung carcinoma on connective tissue explants of rats.

METHOD

For the experiments we used intensively growing connective tissue cultures from newborn rats of the "Wistar" line. Cultivation took place in Carrel flasks by the usual method adopted in laboratories under the direction of A. D. Timofeevskii. During subculture, each explantate was cut in two, and the halves were placed in different flasks. In this way six groups of cultures were obtained, of which one grew without any form of interference in the ordinary nutrient medium. The second group was treated seven times with methylcholanthrene in horse serum in a dose of 1 γ / ml of nutrient medium. The third and fourth groups grew in a medium to which was added on two occasions (at intervals of one month) extracts from two different human lung tumors (in the third group, crude extracts, in the fourth group, extracts heated to 60° for 2 hours which, according to data in the literature, inactivates many viruses). As shown by investigations under the electron microscope, considerable numbers of globular virus-like corpuscles, measuring 50-70 mμ, were found in both tumors. The fifth and sixth groups of cultures were preliminarily treated seven times with methylcholanthrene, and then treated twice with extracts of human lung carcinomas; in the first of these two groups crude extracts were added, and in the second, heated extracts. In our experiments we thus had one experimental group of cultures (methylcholanthrene and crude extract from a carcinoma of the lung) and five control groups. In the process of the investigation a morphological study was made of the experimental and control explantates. The cultures were fixed in copper acetate by Shabadash's method and stained with hematoxylin by Weigert's or Heidenhain's method.

RESULTS

The investigation of living cultures and stained total preparations showed that the first two groups of cultures, grown in an ordinary medium and treated with methylcholanthrene, were almost morphologically indistinguishable from each other (Fig. 1). The effect of

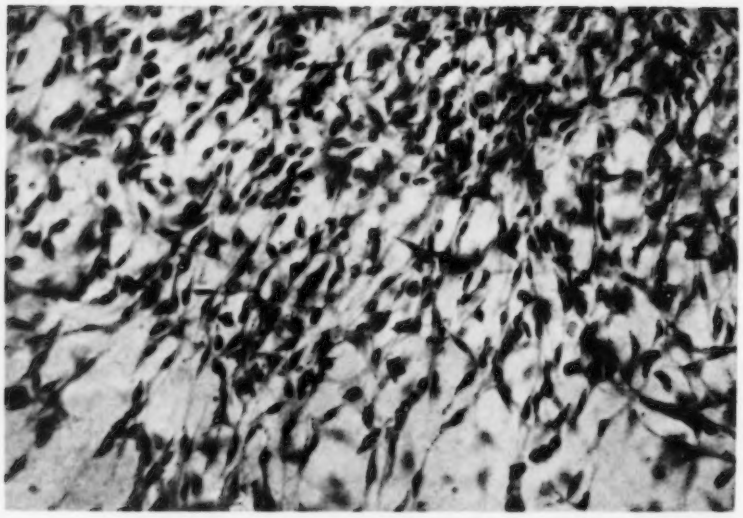

Fig. 1. Zone of growth of a control culture of rat connective tissue (38th transplantation, 12 days). Stained with Weigert's hematoxylin. Magnification 130x.

methylcholanthrene was usually apparent in the first subculture after treatment. In this case growth of the cultures was slightly retarded and in the zone of growth were observed rounded cells and individual disintegrating cells. After transplantation of the cultures into fresh nutrient medium, growth of the explantates was restored, and the zone of growth acquired its usual appearance. After complete cessation of the action of the carcinogen, the cultures grew in the ordinary nutrient medium; their growth became slightly more intensive than that of the cultures not treated in any way. The zone of growth of the explantates became thicker, the cells were more closely packed together, and among them were occasionally observed multinuclear giant cells, although these discoveries were not constant (Fig. 2).

The third and fourth groups of cultures, treated with crude and heated extracts of human lung carcinomas, were morphologically indistinguishable, after observations lasting over 20 months from explantates grown in ordinary nutrient medium and untreated in any way.

Highly significant morphological changes were observed in the fifth and sixth groups of explantates, after the combined treatment with methylcholanthrene and extracts (crude and heated) of human lung tumors. In the first months after treatment the cultures were indistinguishable from the controls, but after four months these cultures showed a more intensive growth; their zone of growth became so thick that it was readily detected with the naked eye.

As a result of the close packing of the cells, the outline of the edge of the zone of growth was clearer and better defined. The individual cells appeared rounder and more flattened; some of them were without processes and, for this reason, they acquired an epithelial-like form. At the edge of the zone of growth giant cells were found, with a homogeneous and, sometimes, foamy cytoplasm. In these cells could be observed a single, hypertrophied nucleus or 4-7 (or more) nuclei, usually not arranged in any definite order, but occasionally in palisade fashion. The nuclei appeared juicy, and regularly contained 3-5 large nucleoli. Most of the cells of the zone of growth showed signs of a considerable polymorphism. Figures of mitotic division were very often found in the cultures, and from time to time these were atypical. Cells in a state of direct division were often found.

At the 10th-12th month of observation, the morphological picture described became more constant and was found in nearly all the cultures. During further cultivation these changes became a regular feature. The zone of growth of certain cultures after growth for 20 months consisted of a collection of giant, multinuclear cells, separated from each other by thin bands of atypical fibroblast-like cells.

The combined action of methylcholanthrene and human lung carcinoma extract thus caused characteristic changes in rat connective tissue cultures, which gave evidence of malignant change in the cultures. The specificity of the morphological changes described was demonstrated by the earlier work of A. D. Timofeevskii, S. V. Benevolenskaya, and M. T. Mogila, showing that the inoculation of cultures with analogous morphological changes is accompanied, as a rule, by the development of tumors in the animals at the site of inoculation. Of some interest is the fact that malignant change occurred in the cultures after the action of heated as well as crude extract. The findings of Gross [21], however,

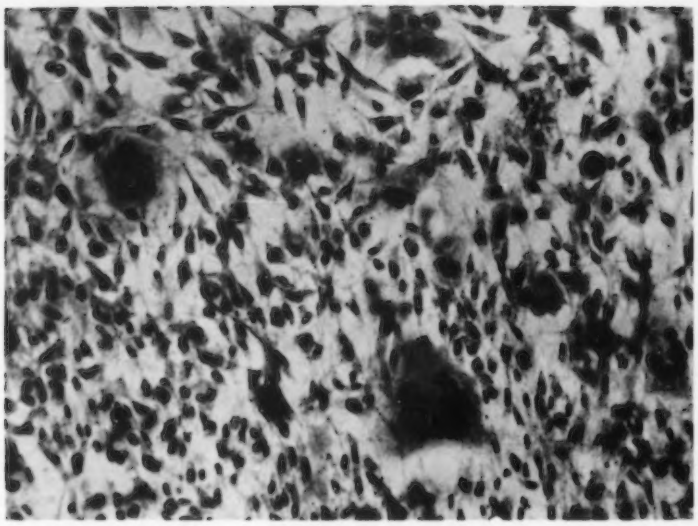

Fig. 2. Zone of growth of a culture of rat connective tissue, treated 20 months beforehand with methylcholanthrene and an extract of a human lung carcinoma. Stained with Weigert's hematoxylin. Magnification 130x.

show that not all viruses lose their activity even after heating at 68°. It may be supposed that, in our experiments, heating for two hours to 60° did not deprive the tumor extract of its biological properties. For this reason the experiments with crude and heated extracts could evidently be regarded as identical.

SUMMARY

The authors studied the morphology of connective tissue explants in rats subjected to the combined effect of methylcholanthrene and extract of cancer tissue of human lung.

In 4 months and more, stable changes of the growth zone structure were revealed, as well as a large number of multinuclear giant cells.

These changes were retained during culture for two years.

It is suggested that malignant change occurred in the connective tissue cultures of rats.

LITERATURE CITED

[1] O. K. Ashmarina, Vopr. Onkol. 2, 2, 211 (1956).

[2] S. V. Benevolenskaya, Arkh. Patol. 5, 7 (1948).

[3] S. V. Benevolenskaya, Med. Zhur. Ukrain. 21, 3, 21 (1951).

[4] S. V. Benevolenskaya, Abstracts of Scientific Proceedings of the Tenth Session of the General Council of the AMN SSSR, (Moscow, 1956), p. 36 [In Russian].

[5] S. V. Benevolenskaya, in: Problems of the Etiology and Pathogenesis of Tumors (Moscow, 1957), p. 55 [In Russian].

[6] R. B. Gragerova and O. K. Ashmarina, in: Problems of the Etiology and Pathogenesis of Tumors (Moscow, 1957), p. 133 [In Russian].

[7] A. M. Eroshkina, A. S. Shubin, and V. G. Proshchenko, in: Problems of the Etiology and Pathogenesis of Tumors (Moscow, 1957), p. 99 [In Russian].

[8] Z. I. Merekalova, G. A. Piskunova, F. I. Leikine, et al., in: Problems of the Etiology and Pathogenesis of Tumors (Moscow, 1957), p. 125 [In Russian].

[9] M. T. Mogila, in: Problems of the Etiology and Pathogenesis of Tumors (Moscow, 1957), p. 59 [In Russian].

[10] G. A. Piskunova, Z. I. Merekalova, V. N. Kolmykova, et al., in: Problems of the Etiology and Pathogenesis of Tumors (Moscow, 1957), p. 115 [In Russian].

[11] V. N. Ponomareva, A. S. Shubin, and L. S. Evseenko, in: Problems of the Etiology and Pathogenesis of Tumors (Moscow, 1957), p. 105 [In Russian].

[12] K. A. Sedov, in: Problems of the Etiology and Pathogenesis of Tumors (Moscow, 1957), p. 111 [In Russian].

[13] O. D. Timofeevskii, Med. Zhur. Ukrain. 24, 2, 21 (1954).

[14] O. D. Timofeevskii and S. V. Benevolenskaya, Med. Zhur. Ukrain. 20, 2, 18 (1950).

[15] O. D. Timofeevskii and S. V. Benevolenskaya, Med. Zhur. Ukrain. 22, 4, 16 (1952).

[16] O. D. Timofeevskii and S. V. Benevolenskaya, Arkh. Patol. 3, 15 (1953).

[17] O. D. Timofeevskii and S. V. Benevolenskaya, in: Problems of the Pathogenesis and Immunology of Tumors (Moscow, 1956), p. 14 [In Russian].

[18] A. S. Shubin, in: Problems of the Etiology and Pathogenesis of Tumors (Moscow, 1957), p. 49 [In Russian].

[19] L. Dmochowski and R. D. Passey, in: The Role of Viruses in the Development of Tumors (Moscow, 1953), p. 220 [Russian translation].

[20] W. R. Earle, J. Nat. Cancer Inst. 4, 165 (1943).

[21] L. Gross, Cancer Res. 18, 371 (1958).

TUMORS INDUCED BY METHYLCHOLANTHRENE IN THE STEPPE LEMMING. A TRANSMISSIBLE STRAIN OF LEMMING SARCOMA (LS)

N. I. Bolonina

Laboratory of Tumor Strains (Head—Dr. Biol. Sci. E. E. Pogosyants) of the Division of Etiology (Head—Active Member AMN SSSR A. D. Timofeevskii) of the Institute of Experimental Pathology and Therapy of Cancer (Dir.—Corresponding Member AMN SSSR N. N. Blokhin), AMN SSSR, Moscow

(Presented by Active Member AMN SSSR A. D. Timofeevskii)

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Recently the steppe lemming (Lagurus lagurus Pall.), a small rodent used for several years in work on tularemia and listeriosis [2], has recently been used as an object for experimental oncological research [1, 3, 4]. Because of its ease and rapidity of multiplication and the simplicity of its care and feeding, as well as its resistance to mouse ectromelia, it has been found to be a convenient laboratory animal. The results of our first experiments [5], in which we used a carcinogenic compound [9:10-dimethyl-1:2-benzanthracene (DMBA)] and heterotransplantation of tumors, show that the steppe lemming is suitable for experimental oncological research.

The aim of this work was to test the sensitivity of the steppe lemming to the action of another carcinogenic compound – methylcholanthrene (MC)—whose carcinogenic properties are less pronounced than those of DMBA. We also aimed to inoculate tumors induced by MC in order to obtain a transmissible strain of the tumor.

METHOD

Experiments were carried out on 32 animals (15 females and 17 males) aged from one to 16 months, which received subcutaneous injections of a 0.5% solution of MC in peach oil in a dose of 0.2 ml. Each animal received 1 mg of the compound. The tumors which developed were inoculated into young lemmings at about one month of age. For inoculation, a suspension of tumor tissue in physiological saline was injected subcutaneously and, in subsequent generations, intramuscularly also

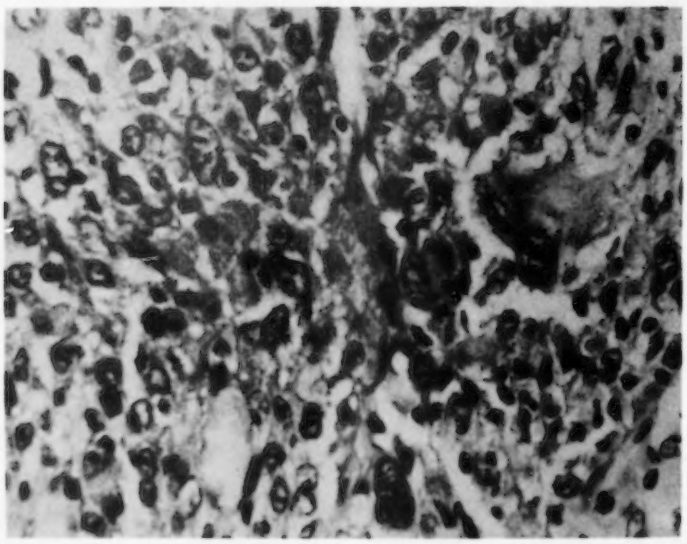

Fig. 1. Polymorphocellular sarcoma in a lemming. Stained with hematoxylin-eosin. Magnification 500×.

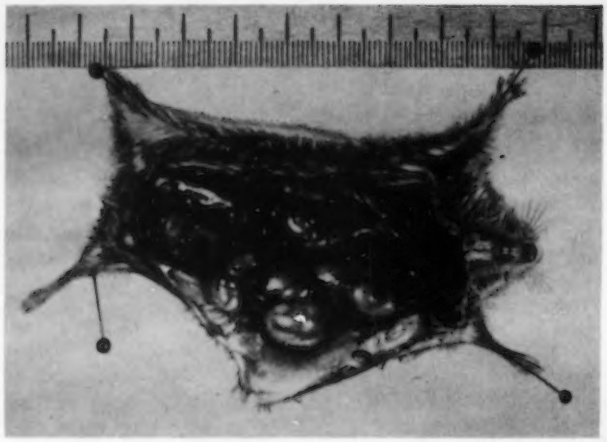

Fig. 2. Tumor, inoculated subcutaneously (first 5 generations).

in a dose of approximately 0.4-0.5 ml. For histological investigation, all the tumors were fixed in 10% formalin and sections were stained with hematoxylin-eosin and by Van Gieson's method.

RESULTS

The first tumors were observed four months after the beginning of the experiment, and they continued to appear throughout the next 11 months. Of the 32 animals taking part in the experiment, 24 survived until the time of appearance of the first tumor. Tumors developed in 13 of these animals (54%). In two animals, tumors developed between four and five months from the start of the experiment, in one after five to six months, in three after six to seven months, in one after seven to eight months, in five after eight to nine months, and finally, in the last after 11 to 12 months. The largest number of tumors (9 of 13) developed between six and nine months. Tumors developed in the animals independently of their sex and initial age. The longest period of survival of lemmings with tumors was two months. This period, however, is possibly not the upper limit, for we sacrificed most of the lemmings with tumors in order to inoculate these.

Histological investigation of the tumors which developed showed that 12 of the 13 were polymorphocellular sarcomas and one was an adenosarcoma. In all the polymorphocellular sarcomas many giant cells and mitoses were seen. No metastases were found in the lymphatic glands, the lungs, or other organs.

On comparison of our findings with those of E. E. Pogosyants [4], who gave lemmings a subcutaneous injection of 0.5 mg of DMBA, it is apparent that the incidence of development of tumors in the first (54%) and second (52.5%) cases was roughly the same. The difference between the action of DMBA and MC was shown only by the fact that, after DMBA, the tumors developed in a shorter period of time. After injection of DMBA the first tumors appeared three months from the start of the experiment, and the largest number of tumors (8 of 12) appeared 4-5 months from the start of the experiment.

Nine of the 13 tumors developing in our experiment were inoculated in other animals. Two of the nine tumors were unsuccessfully inoculated in the first generation. One of these initiated a strain which is described below, while the other was not transplanted further.

Strain LS (lemming sarcoma). The original tumor developed four months from the start of the experiment in an eight-month-old female. The tumor was the size of a large hazelnut, not very firm, with some degree of destruction in the center. In its morphological structure the tumor was a polymorphocellular sarcoma (Fig. 1).

In the first five generations, the tumor was inoculated subcutaneously (Fig. 2). The percentage of successful takes was small (47). After the sixth generation the tumor was inoculated intramuscularly, into the left hind limb. The proportion of successful inoculations by this method was higher, and in order to secure more rapid adaptation, we changed over to intramuscular inoculation.

The percentage of successful inoculations of the tumor by the intramuscular method, investigated for 26 generations, was 82.3. The spontaneous absorption rate was 7%. Only nodules and small tumors were absorbed. No absorption of middle-sized and large tumors was observed. In no case were metastases found.

The earliest time at which death of an animal with a tumor was observed was the 11th day, and the latest the 26th day after inoculation. The average duration of survival of lemmings with tumors was 14-16 days. The tumor was inoculated on the 8th-9th day.

In the 17th generation the tumor was again inoculated subcutaneously, and subsequently the two substrains were inoculated simultaneously—subcutaneously and intramuscularly. After intramuscular inoculation, the rate

of successful subcutaneous inoculation of the tumor, investigated for the first ten generations, was 90%, but the absorption rate of the tumors was much higher (19%) than after intramuscular inoculation. The mean duration of survival of the lemmings with tumors after subcutaneous inoculation was slightly longer (16-20 days).

No significant changes took place in the morphological structure of the tumor in the process of the inoculations.

SUMMARY

It is shown that tumor development in the steppe lemming (Lagurus lagurus Pall.) can be induced by subcutaneous administration of methylcholanthrene (1 mg).

A strain of transplantable polymorphocellular sarcoma of these animals is described.

LITERATURE CITED

[1] N. I. Bolonina, in: Problems of the Etiology and Pathogenesis of Tumors (Moscow, 1957), p. 233 [In Russian].

[2] T. N. Dunaeva, Zhur. Mikrobiol., Epidemiol., i Immunobiol. 9, 51 (1957).

[3] L. V. Ol'shevskaya, in: Problems of the Etiology and Pathogenesis of Tumors, (Moscow, 1957) p. 229 [In Russian].

[4] E. E. Pogosyants, Vopr. Onkol. 2, 2, 193 (1956).

[5] E. E. Pogosyants, and N. I. Bolonina, Vopr. Onkol. 5, 3, 281 (1959).

THE IMMUNOGENIC PROPERTIES OF EXPLANTATES OF BROWN-PEARCE RABBIT TUMORS WHEN CULTIVATED IN HUMAN SERUM

M. S. Lomakin

Laboratory of Noninfectious Immunology (Head—Prof. I. N. Maiskii) of the Institute of Experimental Biology (Dir.—Prof. I. N. Maiskii), AMN SSSR, Moscow

(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

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The study of the character of growth of explantates or heterotransplantates of malignant tumors in animals of the homologous species has contributed greatly to out ideas of immunity against tumors and is, therefore, of undoubted theoretical and practical interest. The experimental treatment of this subject, however, remains incomplete. Certain aspects of the problem are debatable. Some authors [1, 5] consider that malignant tumors, after prolonged heterotransplantation or explantation, remain absolutely unchanged in immunological, biological, and cytological respects, but others, on the contrary, accept the possibility of changes in the antigenic and biological properties of such tumors [2, 4, 6]. Mollendorf [16], for example, inoculated rabbits with explantates of a Brown-Pearce carcinoma, cultivated in a homologous medium, and in a large percentage of cases observed positive results. Similar observations have been made by other authors [10, 15]. Favorite and Cheever [9], in their experiments, observed only 59% of positive inoculations of explantates of a Brown-Pearce rabbit tumor in the animals originally bearing the tumor. There are reports [6, 18] of loss of virulence and a considerable decrease in the number of successful inoculations of malignant tumors in mice after explantation of these tumors in heterogenic nutrient media.

In view of the contradictory findings on this subject, we decided to study the character of the inoculation rate and the growth in the original carriers of the primary tumor after its comparatively prolonged explantation in a heterogenic nutrient medium.

At the same time we attempted to study the reaction of the animals to the inoculated tumor explantates.

METHOD

For the investigations we used a Brown-Pearce rabbit carcinoma, taken for explantation from metastases of this tumor in the internal organs of cancerous animals. The tumor tissue was cultivated in Carrel dishes by the method which we have previously described [3]. The solid phase was chick plasma. The liquid medium consisted of 40-50% human serum, 20-25% chick or rat embryonic extract, 0.5% yeast extract, and a balanced salt solution in which, besides the other ingredients, were included glucose and ascorbic acid. Human serum was used because its action on animal tumors has been adequately studied [13, 14], and it contains a large number of antigens [11, 17]. After cultivation for a predetermined time, the test explantates were taken from the Carrel dishes in sterile conditions, cut into small pieces and made into a suspension in fresh, undiluted human serum, in a volume of 1 ml, which was injected twice into adult chinchilla rabbits, into the same testis, with an interval of 30 days between inoculations. For the first inoculation we used 60-65-day old explantates of tumor tissue and for the second, 30-day old explantates. Animals in which the inoculation of tumor tissue explantates was unsuccessful on both occasions were given an injection of untreated tumor tissue into the same testis on the 10th day, and a proportion of them received, in addition, injections of tissue into the other testis and intraperitoneally. The dose of material inoculated in these cases amounted on the average to 0.5-0.7 ml of a 10% suspension of tumor fragments in physiological saline. Sera were taken from these animals before and after inoculation of the test cultures and also after growth and regression of the untreated tumor tissue, and were tested by the complement fixation reaction for the presence of antibodies against the antigens of the tumor tissue and normal rabbit liver.

RESULTS

Inoculation of rabbits with explantates. During the cultivation of the Brown-Pearce rabbit carcinoma in this medium it was found that not all explantates showed intensive growth. In some cases growth of the test cultures was very intensive and this applied to many of the fragments (Fig. 1), but in others the cultures were in a stage of survival or had zones of growth with solitary cells migrating along the periphery (Fig. 2). Both intensively growing explantates and those in a stage of survival were used for the inoculations.

TABLE 1

Results of Double Intratesticular Inoculation of Rabbits with Explantates of a Brown-Pearce Rabbit Carcinoma, Cultivated in Human Serum

Rabbit No.	Inoculation I			Inoculation II		
	number of cultures which grew well	number of cultures which grew badly	results of inoculation	number of cultures which grew well	number of cultures which grew badly	results of inoculation
1206	2	14	Unsuccessful	4	16	Unsuccessful
1824	1	19	"	5	11	" "
993	3	12	"	6	14	" "
669	5	10	Temporary growth	10	12	" "
1721	1	11	Unsuccessful	8	16	" "
1287	3	14	Temporary growth	5	15	" "
1974	2	13	Unsuccessful	7	15	" "
2956	1	12	"	3	15	" "
1015	1	14	"	5	15	" "
1516	1	10	"	3	14	" "
2434	5	15	Temporary growth	10	15	" "
2591	3	13	" "	8	7	" "
2051	2	11	Unsuccessful	4	17	" "
2877	2	7	"	7	11	" "
3323	3	10	Temporary growth	6	14	" "
2556	4	15	" "	7	15	" "
3724	5	19	" "	7	7	" "
1819	1	17	Unsuccessful	5	15	" "
2195	1	14	"	6	11	" "
3970	4	15	Temporary growth	9	11	" "
3651	3	27	Unsuccessful	5	15	" "
3138	4	26	"	7	13	" "

It is clear from the figures in Table 1 that at the first inoculation, in 14 of the 22 rabbits no growth of the test cultures took place; however, in eight rabbits, firm nodules began to be palpable in the testis on the 7th-8th day, and on the 14th-15th day they reached comparatively large dimensions, but thereafter they began to decrease in size gradually. On the 25th-30th day they had completely regressed. After the second inoculation no growth of the test cultures was generally apparent. The results of these experiments show that explantates of a Brown-Pearce rabbit carcinoma, cultivated outside the body in human serum and the other ingredients of the nutrient medium for two months, and then inoculated mixed with fresh human serum, lose their ability to be successfully inoculated back into the initial carrier of the tumor. This probably took place because we used a small number of viable cultures for the first inoculation. The results of later years, however, show that tumors may develop in animals even when inoculated with a single tumor cell [7]. Our results are in agreement with those obtained on other experimental objects [12, 6].

In subsequent experiments all the experimental rabbits received a third inoculation in the same testis, this time of untreated tumor tissue, whereupon in eight rabbits the inoculation of the Brown-Pearce carcinoma was in general unsuccessful, but in ten rabbits tumor nodules appeared in the testis on the 6th-7th day, and continued to increase in size. Later they began to grow smaller and eventually to regress completely. In one rabbit (No. 1721), a large tumor developed in the testis, and began to infiltrate along the course of the spermatic cord. On the 25th-30th day massive tumor deposits began to be palpable in the peritoneal cavity of this rabbit. Later, on the 60th-65th day, they began to decrease in size and ceased to be palpable on the 90th day. The rabbit was sacrificed on the 98th day. No metastases were found in the organs. Some of the tumor nodules, however, were found to be in a state of complete necrosis on the surface of the peritoneum and in the mesentery of the large and small intestine. In another rabbit (No. 2956), sacrificed on the 93rd day, one completely necrotic metastasis was found in the omentum. In two other rabbits (Nos. 3651 and 3138), after inoculation with untreated tumor, firm nodules began to be palpable in the testis on the 5th-7th day, and on the 12th day they reached a large size. Both rabbits, however, died from accidental infection on the 43rd and 48th days. At necropsy of rabbit No. 3138 no mestastases were found. In rabbit No. 3651, two metastases were

Fig. 1. Living 30-day old culture of Brown-Pearce carcinoma, growing intensively in human serum. Magnification 70 x .

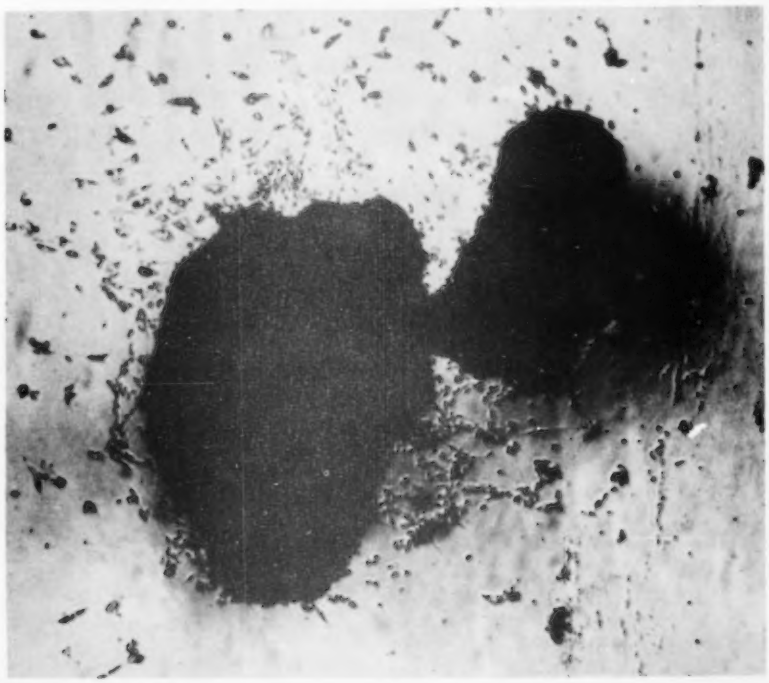

Fig. 2. Thirty-day old culture of a Brown-Pearce carcinoma, cultivated in human serum. Stage of survival. Magnification 70 x .

TABLE 2

Complement Fixation Reaction of the Sera of the Rabbits of the Experimental Group with Antigens from Brown-Pearce Carcinoma and Normal Rabbit Liver

Time of inoculation of tumor	Antigen from Brown-Pearce tumor				Antigen from normal liver tissue			
	dilution of sera							
	1:20	1:40	1:80	1:60	1:20	1:40	1:80	1:60
Rabbit No. 1015								
Before inoculation of tumor	++	+	h	h	++	++	h	h
On the 30th day—first regression	+++	++	+	h	+++	+++	++	h
On the 40th day—second regression	+++	+++	+	h	+++	+++	++	h
On the 52nd day—third regression	+++	++	h	h	++	++	h	h
Rabbit No. 1516								
Before inoculation of tumor	++	h	h	h	++	h	h	h
On the 30th day—first regression	+++	+++	++	h	+++	++	++	h
On the 40th day—second regression	+++	+++	++	h	+++	+++	++	h
On the 52nd day—third regression	++	+	h	h	+++	++	+	h
Rabbit No. 1287								
Before inoculation of tumor	h	h	h	h	h	h	h	h
On the 30th day—first regression	+	h	h	h	h	h	h	h
On the 40th day—second regression	+	h	h	h	h	h	h	h
On the 50th day—third regression	h	h	h	h	h	h	h	h
Rabbit No. 1721								
Before inoculation of tumor	++	h	h	h	++	h	h	h
On the 30th day—first regression	++	+	h	h	++	h	h	h
On the 40th day—second regression	+++	++	h	h	+++	++	h	h
On the 50th day—growth of the tumor	+	h	h	h	h	h	h	h
Rabbit No. 2956								
Before inoculation of tumor	++	±	h	h	++	+	h	h
On the 30th day—first regression	++	+	h	h	++	++	±	h
On the 40th day—second regression	+++	+++	++	h	+++	+++	++	h
On the 50th day—third regression	+++	++	+	h	+++	+++	++	h
Rabbit No. 1974								
Before inoculation of tumor	+	h	h	h	±	h	h	h
On the 30th day—first regression	++	+	h	h	++	h	h	h
On the 40th day—second regression	+++	++	±	h	+++	++	h	h
On the 50th day—third regression	+	h	h	h	+	h	h	h

found in the kidney and one in the omentum, and one of the metastases in the kidney was necrotic, whereas the other contained viable tissue.

Of the 22 experimental rabbits, 18 were thus fully immune to inoculation with untreated tumor tissue (81.8%); in two rabbits the untreated tumor showed definite growth, but thereafter it partially or completely regressed; two rabbits were doubtful, for they died from infection and, moreover, one had a metastasis consisting of viable tissue.

Subsequently nine experimental rabbits were inoculated in the other testis a second time with untreated tumor. In all cases the rabbits were immune to this inoculation too. The other eight experimental rabbits were inoculated a second time in the peritoneal cavity with untreated tumor tissue. The results of inoculation of the tumor in this case too were negative. Twenty-four rabbits were used as controls. In five of the eight rabbits which were inoculated with untreated tumor tissue (62.5%) positive results were obtained, and these rabbits died from tumors on the 28th and 38th days. In three rabbits of this group the tumor showed some degree of growth at first, and then regressed. Of the eight rabbits vaccinated twice at an interval of one month with nutrient medium alone, with explantates, six rabbits died from inoculation of untreated tumor tissue on the 24th and 40th days. Two rabbits were immune. Of the six rabbits inoculated with original tumor tissues, perliminarily

treated for one hour with fresh human serum, two died from tumors on the 22nd and 38th days. The remainder were immune to the inoculation. Finally, two rabbits which received inoculations of a minimum amount of tumor (20 fragments measuring approximately 1 mm^2) died on the 23rd and 38th day. Of the 24 control rabbits, 15 (62.5%) thus died from tumors.

The sera of the experimental and control groups of rabbits were subsequently tested for the presence of antibodies against antigens from the tumor and from normal rabbit liver.

Serological examination of the rabbits' sera. In the experimental group sera were obtained from six rabbits at the following times: a) Before inoculation of the explantates, b) after the first regression of the experimental cultures (30 days), c) after the second regression of the experimental cultures (40th day), and d) after regression of the untreated tumor tissue (50th day). In the control group sera were also obtained from six rabbits at the following times: a) Before inoculation with untreated tumor tissue and b) on the 30th day of growth or regression of the untreated tumor. These sera were tested in the complement fixation reaction with antigens from the tumor and from normal rabbit liver. The test was performed in the usual manner.

It will be seen from the results given in Table 2 that the sera of the experimental group of rabbits, taken before inoculation of the cultures, reacted feebly or not at all with tumor and liver antigens. A somewhat different picture was seen after the first and especially after the second regression of the tumors. In this case the greater part of the sera reacted with tumor and liver antigens in dilution of 1:40 or 1:80 to the degree ++. Only the serum of one rabbit (No. 1287) did not react with these antigens in any dilutions.

In the control group only one serum, taken from a rabbit after regression of the tumor, reacted with antigens from tumor and liver in dilution of 1:40 to a degree ++. The remainder of the sera did not react with these antigens in these dilutions, or they reacted like the sera taken from the rabbits before inoculation with the tumor. The results of these experiments show that explantates of a Brown-Pearce rabbit carcinoma, cultivated in human serum, possess definite immunogenic properties. It is very probable also that the appearacne of antibodies in the blood of the rabbits after regression of the explantates is also due to the components of the nutrient medium in which the experimental cultures were grown. These components were heterogenic embryonic extract, human serum, and yeast extract.

SUMMARY

The explants of a Brown-Pearce rabbit carcinoma cultured on human serum and heterogenic embryonic and yeast extract, show growth in a number of cases, when mixed with fresh undiluted human serum and transplanted intratesticularly in rabbits; later they regress.

Rabbits in which the experimental cultures have regressed twice in the same testis became immune to repeated transplantation of the untreated tumor tissue into the same testis. In these rabbits, antibodies to the tumor and normal rabbit liver are produced more intensively than in the control group.

LITERATURE CITED

[1] A. I. Ageenko, Vopr. Onkol. 3, 2, 169 (1957).

[2] M. S. Lomakin, Byull. Eksptl. Biol. i Med. 36, 6, 65 (1953).

[3] M. S. Lomakin, Byull. Eksptl. Biol. i Med. 47, 5, 91 (1959).•

[4] I. N. Maiskii, The Biological Basis of Immunity Against Cancer (Moscow, 1955) [In Russian].

[5] V. N. Stepina, Heterogenic Inoculation of Brown-Pearce Tumor and the Study of the Antigenic Properties of the Heterotransplantates. Candidate's dissertation (Moscow, 1955) [In Russian].

[6] C. J. Dawe, M. Potter, and J. Leighton, J. Nat. Cancer Inst. 21, 753 (1958).

[7] P. S. DeRopp and D. McKenzie, Cancer Res. 14, 588 (1954).

[8] W. R. Earle, E. Shelton, and E. L. Schilling, J. Nat. Cancer Inst. 10, 1105 (1950).

[9] G. O. Favorite and F. S. Cheever, Cancer Res. 136 (1941).

[10] A. Fischer and F. Davidsohn, Nature 143, 436 (1939).

[11] P. G. H. Gell, J. of Clin. Pathol. 8, 269 (1955).

[12] J. Kieler, Rev. Hemat. 10, 516 (1955).

[13] C. Landschutz, Ztschr. Naturforsch. 9, 406 (1954).

[14] C. Landschutz, Naturforsch. 11, 304 (1956).

[15] W. H. Lewis, Science 81, 545 (1935).

[16] M. Mollendorf, Arch. exper. Zellforsch. 21, 411 (1938).

[17] I. Oudin, J. Immunology 81, 376 (1958).

[18] K. Sanford, G. Hobbs, and W. Earle, Cancer Res. 16, 162 (1956).

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THE PRODUCTION OF SPECIFIC SERA TO EHRLICH'S MOUSE CARCINOMA ON THE BASIS OF THE PHENOMENA OF ACQUIRED IMMUNOLOGICAL TOLERANCE

Yu. N. Mol'kov

Laboratory of Experimental Biotherapy (Head—Corresponding Member AMN SSSR M. M. Maevskii) of the Institute of Experimental and Clinical Oncology (Dir.—Corresponding Member AMN SSSR N. N. Blokhin), AMN SSSR, Moscow

(Presented by Active Member AMN SSSR A. D. Timofeevskii)

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The problem of the production of monospecific antisera against malignant tumors has interested many researchers in recent years. Nearly all the methods described for producing monospecific sera are based on in vitro methods of absorption of nonspecific antibodies, and their results are not very satisfactory. Nevertheless, the production of a monospecific antiserum to individual species or groups of human malignant tumors would possibly be of both practical and theoretical importance, especially in the development of the immunological diagnosis of cancer.

Several authors [4, 11-13] have expressed the opinion that it is possible to obtain "pure" specific antisera by means of the method of artificial tolerance. These workers have also reported having obtained monospecific sera against certain antigens of normal and tumor tissue in animals on the basis of the phenomenon of acquired immunological tolerance.

In this communication we describe the results of experiments to produce a monospecific antiserum to Ehrlich's mouse carcinoma.

When planning this investigation, we set out from the position that animals (rabbits), when treated with foreign antigens (normal mouse tissues) in the first days of postembryonic development or in the period of embryogenesis, if given further immunization in the adult state with the tumor tissue, will not form specific antibodies to the normal tissue antigens but, at the same time, retain the power fully to form antibodies to the specific tumor antigen occurring in tumor tissue in conjunction with the normal tissue antigens.

Modern development in methods of creating immunological tolerance have encouraged the hope that it may be possible to differentiate by this means between different types of antigens and antibodies [1-3, 5, 9, 10, 14, 15].

METHOD

Investigations were carried out on female chinchilla rabbits, weighing about 3 kg and kept on an ordinary diet.

As normal tissue antigens we used a mixture of tissues, consisting of citrated whole blood, liver, spleen, and kidney, and also tissue from whole embryos of healthy adult white mongrel mice, which usually supports the ascitic strain of Ehrlich's carcinoma. This mixture of tissue antigens was prepared from organs taken from not less than ten mice.

The normal organs of the mice (liver, kidney, spleen, embryos) were ground in a sterile mortar with glass. From them we prepared a 50% suspension of the tissues in physiological saline, to which, before injection, was added citrated whole blood in a proportion of 25% of blood to the suspension of tissue cells of the organs. Whole blood from healthy white mongrel mice was collected in a 1% solution of sodium citrate in order to give a 90% suspension of whole blood in the citrate solution. After being ground, the antigen for intravenous injection into the female animals was centrifuged and the supernatant fluid was used, to which was added citrated whole blood in order to obtain a 50% suspension of citrated whole mouse blood. Altogether, each female received 15 ml of antigen intravenously (5 ml per injection). Antigen was injected on the 23rd-25th and the 27th day of embryogenesis.

The newborn rabbits usually received subcutaneous and intraperitoneal injections of a mixture of normal antigens starting on the first to the fifth day after birth, in a total dose of 1.5 ml (0.3 ml daily for 5 injections).

Antigen was injected into the embryos on the 17th day of embryogenesis. Laparotomy was performed on the females through a midline incision. Without opening the uterus, an injection of 0.2 ml of the antigen mixture was given to each embryo in the region of the spine. In

order to prevent the development of postoperative infection, 100,000 i. u. of crystalline penicillin dissolved in 5 cc of physiological saline was injected into the abdominal cavity. The operation wound was closed in layers, painted with collodion, and covered with a sterile gauze dressing. It should be mentioned that a large proportion of the embryos subsequently died and underwent absorption, and a number were born dead. Thus, it was only possible to obtain a small percentage (10-15) of living, intact young rabbits, suitable for carrying out the subsequent experiments.

In one series of the experiment, antigen was injected not only into the newborn rabbit but also into the mother during the second half of embryogenesis. In doing so, we worked on the assumption that antigens, injected intravenously in large quantities into the mother, would come into contact with the embryos. Several authors [14] have shown that it is possible to obtain tolerant rabbits after the injection of antigen by this method. After the preparation of standard solutions of antigens, the total protein content was determined in milligrams per milliliter of solution by Kjeldahl's micromethod. Thus, we could calculate the quantity of protein injected into each experimental rabbit.

When they attained the age of ten weeks, the rabbits were immunized with tumor tissue. For immunization we used undiluted ascitic fluid from an Ehrlich's carcinoma. In order to obtain specific tumor antisera rapidly, we used the standard scheme of immunization that we usually employ [8].

To determine the specificity of the sera obtained, we used the hemagglutination reaction with normal mouse red cells and also with red cells from mice with the ascitic form of Ehrlich's carcinoma. As tumor antigen we used blood taken from mice with an Ehrlich's carcinoma in an advanced stage of the disease, when abundant metastases were usually present, as revealed by postmortem examination of the animal at the time of taking the blood. The presence of carcinoma antigen in the blood of these mice could always be revealed by the specific hemagglutination reaction with tumor antisera, a detailed description of which we have given previously [6, 7, 8].

As a control of the reliability of the results obtained by the hemagglutination reaction we also used the specific precipitation reaction in agar, a description of which is given in our previous communications [6, 7, 8]. As antigens for use in the precipitation reaction in agar we used: normal blood and blood from mice with an advanced stage of carcinoma, the solid tumors and ascitic fluid of Ehrlich's carcinoma, and normal mouse tissues (spleen, liver, and kidney).

RESULTS

In order to obtain specific antitumor sera against Ehrlich's mouse carcinoma on the basis of the phenomenon of an artificially created immunological tolerance, we tested a number of schemes of production of immunological tolerance in rabbits to heterogenic cell antigens.

We investigated the possibility of obtaining immunological tolerance by injection of antigen: 1) Into newborn rabbits from the first to the fifth day of life (each young rabbit received 130.5 mg of protein); 2) intravenously into the mother in the last days of embryogenesis (each female received 2325 mg of protein) followed by supplementary injection into the newborn animals (130.5 mg to each young rabbit); 3) into the embryos on the 17th day of embryogenesis (17.4 mg of protein to each embryo); 4) into the embryos (17.4 mg of protein) with a supplementary injection into the new-born animals (130.5 mg of protein).

The experimental results are shown in summarized form for the different groups in the table.

It is clear from the results of the hemagglutination reaction that a true decrease was observed in the formation of normal hemagglutinins in the experimental rabbits by comparison with the controls in the second, third, and fourth groups, and especially in the third and fourth, in which an injection of antigens of normal tissues was given into the embryos (third group) and into the embryos, followed by a supplementary injection after birth. The difference between the mean values of the titers of the hemagglutination reaction in the experimental and control animals in these groups is statistically significant. It should also be mentioned that individual tumor antisera in the test groups reacted more intensively with the blood of mice affected by carcinoma.

The sera of these groups of experimental animals thus showed a significant fall in the formation of normal hemagglutinins and a more intensive reaction of individual tumor antisera with the blood of cancerous mice than with the blood of healthy mice. Unfortunately, it was not possible to rid it completely from the formation of normal antibodies.

In regard to nearly all the groups, especially the third and fourth, an interesting phenomenon must be mentioned which was observed during immunization of the rabbits. Beneath the skin in the experimental animals, at the site of injection, the temporary development and temporary (occasionally intensive) growth of tumor tissue was observed, which did not occur in the control rabbits. This fact undoubtedly shows some degree of immunological approximation of the tissues of the experimental animals towards Ehrlich's mouse carcinoma.

The sera of the experimental and control rabbits of all the groups were also tested by the specific precipitation reaction in agar, to test the validity of the results obtained, and identical results were found. The results of the precipitation reaction in agar showed that the tumor antisera obtained from the experimental rabbits were actually more specific than the sera from the control rabbits. The majority of antisera from the third and

TABLE

Mean Titers of the Hemagglutination Reaction in the Experimental and Control Rabbits in the Different Groups of Experiments

Experimental group	Serial No. of rabbit	Titer of natural hemagglutinins		Titer of immune hemagglutinins	
		with blood of healthy mice	with blood of cancerous mice	with blood of healthy mice	with blood of cancerous mice
1st experimental	1	–[1]	–	64[2]	64
	2	–	–	32	64
	3	–	–	64	64
	4	–	–	128	128
1st control	5	–	–	128	128
	6	–	–	256	256
	7	–	–	128	128
	8	–	–	128	128
2nd experimental	1	–	–	128	128
	2	–	–	64	64
	3	–	–	64	128
	4	–	–	32	128
	5	–	–	64	64
	6	–	–	64	128
	7	–	–	128	128
	8	–	–	64	128
2nd control	9	–	–	128	256
	10	–	–	256	256
	11	–	–	256	256
	12	–	–	128	128
	13	–	–	1024	1024
	14	–	–	128	128
3rd experimental	1	2	2	32	64
	2	–	–	32	32
	3	8	8	64	64
	4	–	–	16	32
3rd control	5	–	–	128	256
	6	–	–	128	128
	7	–	–	128	128
	8	–	–	256	256
4th experimental	1	–	–	8	64
	2	–	–	128	128
	3	–	–	64	64
	4	8	8	64	64
	5	32	32	64	64
	6	–	–	64	64
	7	–	–	64	128
	8	–	–	32	32
4th control	9	–	–	256	256
	10	–	–	128	128
	11	–	–	1024	1024
	12	–	–	256	512
	13	–	–	256	512
	14	–	–	512	512
	15	–	–	128	128
	16	–	–	512	512

(P > 0.06) (P ≥ 0.001) (P > 0.000) (P < 0.01)

[1] Absence of hemagglutination reaction

[2] Final dilution of sera, where a hemagglutination reaction is still present.

fourth experimental groups, and also individual antisera from the second group did not react with normal antigens (liver, kidney, spleen, blood, and embryos) even in the initial dilutions, whereas they continued to react with tumor antigen. The tumor antisera of the control rabbits reacted to an equal degree with both normal and tumor antigens.

In the second, third, and fourth groups of experimental rabbits it was thus possible to attain only a slight degree of immunological approximation towards the heterologous mouse antigen. This immunological approximation was incomplete and showed itself only as a slight fall in the formation of normal antibodies and the more intensive growth of Ehrlich's carcinoma tissue, injected subcutaneously during immunization, in the experimental rabbits than in the controls.

In conclusion it should be pointed out that the antisera which we obtained on the basis of the phenomenon of acquired immunological tolerance were, in our opinion, more specific than those obtained by the ordinary method of immunization of the control rabbits.

SUMMARY

The author attempted to obtain specific antitumor sera on the basis of the phenomenon of an artificially created immunological tolerance. It was shown that only an insignificant degree of immunological approximation can be attained in rabbits with respect to heterologous mice antigens by administering the latter to the embryos, especially when this is supplemented by a subsequent inoculation at an early period of extra-uterine life. This immunological approximation is incomplete and is manifested only by an insignificant reduction in the formation of normal antibodies, inadequate for obtaining "pure" monospecific and antitumor sera to Ehrlich's mouse carcinoma. The antisera to the Ehrlich's carcinoma obtained on the basis of acquired immunological tolerance were more specific than those derived by the usual method of immunization in control rabbits.

LITERATURE CITED

[1] M. Gashek, "Problems of vegetative hybridization and tissue transplantation in warm-blooded animals." Obshchaya Biol. 16, 5, 405 (1955).

[2] M. R. Gashek, Abstracts of Proceedings of the First All-Union Conference on Tissue Incompatibility and Transplantation of Organs and Tissues (Moscow, 1957) p. 3 [In Russian].

[3] V. Gashkova, Abstracts of Proceedings of the First All-Union Conference on Tissue Incompatibility and Transplantation of Organs and Tissues (Moscow, 1957) p. 4 [In Russian].

[4] L. A. Zil'ber, I. N. Kryukova, N. V. Nartsisov, et al., Vopr. Onkol. 4, 3, 268 (1958).

[5] L. A. Kozlovskaya, The Origin of Antibodies Present in Offspring of Immunized Mothers. Authors abstract of dissertation (Moscow, 1958) [In Russian].

[6] Yu. N. Mol'kov, Abstracts of Proceedings of the Second Congress of Oncologists and the Third Congress of Roentgenologists and Radiologists of the Ukr. SSR (Kiev, 1956) p. 24 [In Russian].

[7] Yu. N. Mol'kov, Abstracts of Proceedings of a Scientific Conference on Technical Problems of the Immunology of Malignant Neoplasms (Moscow, 1956) p. 6 [In Russian].

[8] Yu. N. Mol'kov, in: Problems of the Etiology and Pathogenesis of Tumors (Moscow, 1957) p. 167 [In Russian].

[9] A. V. Puza, Abstracts of Proceedings of the First All-Union Conference on Tissue Incompatibility and Transplantation of Organs and Tissues (Moscow, 1957) p. 63 [In Russian].

[10] G. Ya. Svet-Moldavskii, Abstracts of Proceedings of a Conference of Young Scientific Workers of the Institute on the Experimental Study of Bacterial Preparations (Moscow, 1957) p. 17 [In Russian].

[11] G. Ya. Svet-Moldavskii and Yu. Z. Gendon, Vopr. Onkol. 4, 3, 263 (1958).

[12] M. Feldman and D. Yaffe, Nature 179, 1353 (London, 1957).

[13] M. F. Woodruff and L. O. Simpson, Brit. J. Exper. Path. 36, 494 (1955).

[14] R. Hanan and J. Oyama, J. Immunol. 73, 49 (1954).

MORPHOLOGICAL CHANGES IN REGENERATING RAT KIDNEY AT LONG INTERVALS AFTER INJURY

G. G. Samsonidze

Laboratory of Growth and Development (Head—Prof. L. D. Liozner) of the Institute of Experimental Biology, AMN SSSR (Dir.—Prof. I. N. Maiskii) and the Department of Histology (Head—Prof. S. E. Sakvarelidze), Tbilisi Medical Institute

(Presented by Active Member AMN SSSR V. V. Parin)

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In previous publications [2, 3] it has been shown that after partial resection (from $^1/_3$ to $^1/_2$) of one kidney and complete removal of the other, restoration of the partially resected organ occurs. Regeneration of the kidney is a complex process [1] involving changes both at the injured surface and in the rest of the kidney, which is not directly affected by the injury (regeneration hypertrophy). We observed restorative processes over a 7-month period.

In the present study we followed the restorative processes for longer periods following injury, since changes in regenerating organs over long periods after injury have not been adequately studied.

METHOD

Experiments were performed on white male rats weighing 150-200 g. The left kidney was removed from each of 10 experimental animals, and $^1/_3$-$^1/_2$ of the right kidney was resected at the same time. Five rats served as controls. The convex portion opposite the hilum was removed in resection, by means of an incision parallel to the long axis of the organ. The part removed contained mainly cortical substance.

After the animals were sacrificed the kidneys were weighed, measured, and fixed in Bouin's fluid and in 10% formalin. Small pieces were imbedded in paraffin, and sections were stained with hematoxylin by Bemer's method, and with azocarmine and hematoxylin by Heidenhain's method.

The area of a median cross-section of the kidney and the areas occupied by cortical and medullary substance in this section were measured.

The areas of individual renal corpuscles were also determined, and the mean area for 50 corpuscles was calculated. In addition, the diameters of the convoluted tubules and collecting ducts and the heights of the tubule cells and renal corpuscles were measured by means of an ocular micrometer. For each of these quantities the mean of 50 measurements was calculated. Statistical analysis of the data showed that this number of measurements ensures that reproducible results will be obtained.

RESULTS

Table 1 presents data characterizing the changes in weight and size of the operated kidneys 15 months after operation, in comparison with results of previous experiments [2, 3] in which the changes were observed 3-7 months after operation.

Data for the control rats were obtained 15 months after the beginning of the experiment. The values found for these rats were not statistically different, in most cases, from those for control rats sacrificed after only three months. Consequently, in the further analysis of our data we proceeded on the basis that during the period between 3 and 15 months no significant morphological changes took place in the kidneys of control rats, and we confined our attention to comparison with a single control.

Taking this into account, we may note that in contrast to the kidneys of control animals, those of experimental animals showed significant changes, which indicated that restorative processes were occurring, during periods long after the beginning of the experiment. The weights of the kidneys of experimental animals were 57% greater after 15 months than after 3 months. The relative weight particularly increased; it was more than twice as great for the experimental as for the control group. This indicated, to be specific, that the experimental animals did not grow as much as the controls. It is interesting that 15 months after the operation the width of the kidney was markedly increased, although just this dimension was reduced by the operation. As a result, the kidneys assumed a form similar to their original form, and the defect was erased to some extent. The area of the median section of the kidney was also significantly greater after 15 months than after 3 months.

TABLE 1

Weights and Dimensions of Right Kidneys of Experimental and Control Rats at Various Intervals After Start of Experiment

Time of examination	Weight of kidney		Dimensions of kidney (in mm)			Area of median section (in mm^2)	
	mg	% of body weight	length	width	thickness	total	cortex
After 3 months	1 552	0,63	21,5	8,7	10,3	56,2	50,5
After 7 months	1 756	0,65	23,2	9,0	10,8	58,4	53,1
After 15 months	2 439	0,93	23,5	13,1	11,8	63,7	58,7
Control	1 394	0,41	18,6	10,6	8,8	44,6	39,8

TABLE 2

Dimensions of Renal Corpuscles and Convoluted Tubules of Experimental and Control Rats

Time of examination	Renal corpuscles						Convoluted tubules					
	total number in section	number per mm^2	area (in μ^2)	maximum dimension of cavity (in μ)	number of nuclei in outer wall of Bowman's capsule	distance between nuclei (in μ)	diameter (in μ)	diameter of lumen (in μ)	area occupied by cells (in μ^2)	number of nuclei per cross-section	distance between nuclei (in μ)	height of cells (in μ)
After 3 months	139	2.8	6,206	8.2	18.6	13.1	46.5	21.9	1,716	7.2	9.4	11.6
After 7 months	147	2.8	6,563	7.1	19.5	12.6	43.6	16.0	1,437	8.1	9.0	12.0
After 15 months	128	2.3	11,387	6.6	21.9	11.7	44.0	12.0	1,796	8.9	8.7	14.1
Control	170	4.3	5,093	4.2	12.9	9.3	35.1	6.1	1,199	4.7	7.7	11.1

The changes in various parts of the kidney, as revealed by microscopic examination, are shown in Table 2.

As is evident from the data in Table 2, the number of renal corpuscles in the regenerating kidneys is less than normal. Thus, the number of renal corpuscles is not completely restored by the regeneration process. The area of the corpuscles is a different matter, especially if the increased dimensions of the kidney are taken into account. It is true that the area of each corpuscle increases even in the kidneys of control animals. It increases from 4338 to 5093 μ^2 during the experiment – i.e., it parallels the age of the animal. This increase is statistically significant (P = 0.01). But in the experimental rats the incant (P = 0.01). But in the experimental rats the increase in corpuscular area is significantly greater than that due to age. This increase is particularly marked in the last five months, when the corpuscle area increases by 73%. The increase in corpuscle area is not connected with an increase in the width of the space between capsule and glomerulus. The latter dimension falls in the late experimental periods, although it remains greater than for the control rats. Along with this, the number of nuclei in the outer wall of Bowman's capsule increases; this increase is statistically significant (P = 0.003).

The changes in the renal tubules are of a different sort from those in the corpuscles. It was not possible to detect further hypertrophy of the tubules as the experiment continued. In fact, the tubule diameters even decreased somewhat, from 46.5 to 44 μ; but in this case the difference was not statistically significant. The reduction in the tubule diameter was accompanied by a considerable diminution in the size of the tubule lumen (from 21.9 to 12 μ), while the area occupied by cells—i.e., the area of the tubular wall—increased (from 1716 to 1796 μ^2).

We previously observed [2] that during the first three months following injury, an increase in tubule diameter occurs in the regenerating kidney, which is partially explained by an increase in the diameter of the lumen. As is apparent from the data obtained in the present study, the changes occurring in the tubule in subsequent experimental periods are different in character. A certain amount of change in the form of the cells in the tubule wall must be noted. The distances between the nuclei of those cells change slightly, becoming 8.7 instead of 9.4 μ. The height of the cells increases considerably, from 11.6 to 14.1 μ; this change is statistically significant (P = 0.000). There are more cells per tubule cross-

section, to judge from the fact that the number of nuclei found in a tubule cross-section increases from 7.2 to 8.9; this increase is statistically significant (P = 0.005). It will be recalled that in the control rats no such changes could be observed. These data refer to the convoluted tubules. We found similar changes in the collecting ducts of the cortex and medulla of kidneys that had regenerated, and therefore we did not make a separate study of these data.

Our results permit us to conclude that in the injured kidney, where about $\frac{1}{3}$ predominantly cortical substance was removed, accompanied by removal of the opposite kidney, restorative processes continue for long periods after injury (from the third to the 15th month after operation). The weight and size of the regenerating kidney increase, and its form changes. Apparently the restorative processes take place primarily in the renal corpuscles, which undergo considerably hypertrophy. As for the renal tubules, their diameters are unchanged; as a result, it is hard to see how they could contribute to the change in the kidney size. It is possible that the tubules increase in length, since we did not take the length of the tubules into consideration in our experiments. But this is unlikely, since the increase in cross-sectional area of the kidney was caused exclusively by an increase in the area of its cortical layer. In view of this we must suppose that the area occupied by the collecting ducts in the medulla does not increase, and since the changes in the collecting ducts are of the same type as those in the convoluted tubules, we can suppose that the convoluted tubules do not increase in length. This argument should be tested experimentally. It should be noted that at the long-time intervals, changes occur in the renal tubules. They take the form of an increase in the number of cells in the tubule wall and a thickening of this wall, with a resulting decrease in the diameter of the lumen. We are justified in regarding these changes as restorative.

Further analysis shows that these changes occur as a result of an increase in the number of cells—i.e., cellular hyperplasia. Cell dimensions do not diminish during the experiment. Cells in the kidneys of experimental animals remain hypertrophied—their dimensions are greater than those of the corresponding cells in the control animals. This shows that the phenomenon of cellular hypertrophy may be of a lasting and stable character.

SUMMARY

Complete removal of the left kidney and resection of $^1/_3$ to $^1/_2$ of the right kidney were performed simultaneously in mature rats. The experiment then lasted for 15 months. Regenerative processes continued long after injury, as shown by comparison with the changes observed 3 months and 8 months after operation. The size and weight of the kidney increased. This increase results from enlargement of the renal corpuscles. The lumen of Bowman's capsule becomes smaller, while the thickness of its outer wall increases. The diameter of the convoluted tubules and collecting ducts does not increase, but the thickness of their walls does, causing a reduction in the size of the lumen. The cells in the tubular wall become taller. These restorative processes occur as a result of cellular hyperplasia, and the cells are larger than in control animals.

LITERATURE CITED

[1] M. A. Vorontsova and L. D. Liozner, Physiological Regeneration (Moscow, 1955) [In Russian].

[2] G. G. Samsonidze, Morphophysical Analysis of Renal Regeneration After Injury (Tbilisi, 1958) [In Russian].

[3] G. G. Samsonidze, Byull. Eksper. Biol. i Med. 6, 101 (1959).*

*Original Russian pagination. See C. B. Translation.

MORPHOLOGICAL REACTION OF UTERINE WALL TISSUES OF THE WHITE RAT TO A FOREIGN BODY

Liu Tsien-Ch'ang

Department of Embryology (Head—Prof. B. P. Tokin), A. A. Zhdanov Leningrad State University

(Presented by Active Member AMN SSSR V. V. Parin)

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At present, the immunity of embryos is receiving considerable attention [2]. Various immunologic reactions (phagocytic, inflammatory, and others) may be of great importance in form-producing processes—in the course of embryonic development and in regeneration phenomena.

Observations [1] on changes in the reactivity of uterine tissues of the mouse in experimentally produced aseptic inflammation show that a great deal remains to be explained about the possible role of inflammatory and phagocytic phenomena in the morphological and physiological rebuilding of uterine tissue.

In the present study we investigated inflammatory and regenerative phenomena in uterine wall tissue of 93 rats in various physiological conditions and at various ages

METHOD

The investigations were carried out on 12-day- and $1^1/_2$-month-old rats, on sexually mature rats in estrus, on pregnant rats (7-14 days), and on rats immediately postpartum.

A foreign body—sterile cotton thread soaked with carmine—was inserted into the wall of the uterus. Insertion of this foreign body, which passed through all the layers of the uterine wall, made it possible to reveal the characteristics of the morphological reactions of its various tissue systems.

The experimental animals were killed at 10 minutes, 1, 3, 6, 12, and 16 hours, and 1, 3, 5, 7, and 10 days after the operation.

RESULTS

Insertion of a foreign body into the uterine wall causes destruction of adjacent tissues and extravasation of blood. During the first hours after injury, two morphologically distinguishable zones are formed: a zone of compressed tissue around the wound canal, and an edematous zone which shades gradually into relatively unchanged tissue. This separation into zones remains for 24 hours.

Beginning with the first hour, and sometimes three to six hours after operation, there is a migration, primarily of specialized granulocytes, out of the blood vessels and a movement of these toward the foreign body. After 12-16 hours, macrophages of Maximov's large lymphocyte type are seen, and the number of these increases gradually. Carmine particles introduced with the thread are very quickly phagocytosed by the specialized granulocytes. A small fraction of the dye is found in the macrophages, but on the third to the fifth day some carmine granules are seen in the fibroblasts. The phagocytes with carmine granules in the wall of the uterus are transported in the direction of the broad ligament and are gathered in groups. A similar picture has also been observed by other authors [6]. Henderson [5] has described movement of phagocytes, predominantly macrophages, out of the uterine cavity into the mucosa and into lymphatic formations under the serosa. In some preparations we have been able to see a transfer of individual specialized granulocytes containing carmine, out of the uterine cavity and beneath the epithelial layer.

After 24 hours, a significant fraction of the leukocytes has undergone degenerative changes, and some of the carmine granules are found outside the cells. On the third day after insertion of the foreign body, fibroblastic elements have accumulated around the thread. By the seventh day, giant connective tissue cells are distinctly visible around the fibers of the thread. On the tenth day a significant hypertrophy of all layers of the uterine wall is noted, and a large number of eosinophiles; numerous giant cells, a layer of fibroblasts, and also individual phagocytes have accumulated around the fibers of the inserted thread. Carmine granules are often visible in the giant connective tissue cells.

Analysis of our data showed that in the white rat the rate of phagocytic and regenerative processes in the uterine wall is different at various physiological conditions.

Determination of the phagocytic activity of the specialized granulocytes in the uterine wall during the first

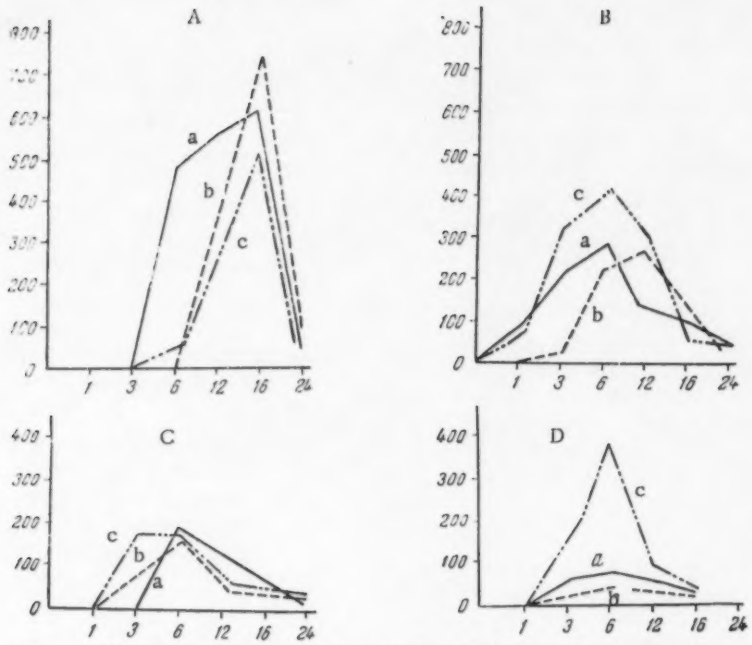

Figure. Changes in the phagocytic activity of specialized granulocytes in tissues of the uterine wall in the white rat. A) 12-day-old rats; B) sexually mature rats (in estrus); C) pregnant rats (on the 7th day); D) immediately post-partum. a – In the uterine cavity; b – in the deep portions of the uterine wall; c – in the uterine serosa. Ordinate) number of phagocytes per 1000 leukocytes; abscissa) hours after operation.

24 hours after insertion of the foreign body resulted in the data shown in the figure.

In the 12-day-old rats (A), the first phagocytes appear three hours after operation. The most intensive phagocytic activity is found after 16 hours. At this time almost all the specialized granulocytes contain carmine granules. After 24 hours many of the specialized granulocytes are dead, and the carmine granules are found outside the cells. It should be noted that in the 12-day-old rats, macrophages showing considerable phagocytic activity accumulate in large numbers around the focus of injury significantly earlier than they do at other ages.

In sexually mature rats (B) during estrus, the transport of specialized granulocytes out of the vessels and the phagocytosis of foreign particles begins earlier than in the 12-day-old rats. Maximum phagocytic activity begins six hours after insertion of the foreign body. Macrophages appear after 24 hours, and in small quantity.

During the period of maximum phagocytic activity, the specialized granulocytes phagocytose only about half of the leukocytes present at the focus of aseptic inflammation.

In mice and rats during estrus, leukocytes in the uterus intensively phagocytose spermatozoa [3]. The introduction of infected sperms into the rabbit uterus during estrus does not cause infection, whereas similar experiments carried out during pseudo-pregnancy do cause infection [4]. The uterus of the cow is resistant to the introduction of a culture of Corynebacterium pyogenes at the time of estrus, but is not resistant when a corpus luteum is present. In this connection it is interesting to compare our data on the phagocytic activity of specialized granulocytes in estrous rats and in pregnant rats. In the figure (C), we find that although in pregnancy the first phagocytes appear, just as in estrus, one to three hours after insertion of the foreign body, and maximum phagocytic activity is observed after six hours, the total number of phagocytes is still very small. Immediately postpartum, phagocytic activity is still further reduced, particularly in the wall of the uterus, although the total number of leukocytes expelled from the blood vessels is extremely large.

The rate of formation of the connective tissue capsule, like the rate of appearance of giant cells, is different in the various stages of ontogenesis. These processes occur very rapidly in 12-day-old rats. In most cases, restoration of a considerable portion of injured tissue has taken place by the third day. Giant cells have appeared around the thread fibers by the fifth day, whereas in sexually mature rats the giant cells are not observed until the seventh to tenth day after operation, and restoration of injured regions and "organization" of the focus of injury occur on the seventh day after the beginning of the experiment. But in analyzing the causes of the more rapid regeneration in 12-day-old rats, we must take into account not only the high phagocytic activity of the specialized granulocytes and macrophages, but also the numerous erythrocytic extravasations that are observed in

the uterine wall when it is injured in various ways, especially in rats of this age. In the vicinity of the extravasated erythrocytes we see lysis of tissue elements, and a considerable disintegration of tissues, which rather quickly is replaced by restoration of the injured areas.

Immediately postpartum, regenerative processes in the uterine wall are somewhat accelerated in comparison with those in nonpregnant animals, but they proceed under such special conditions that it is difficult to analyze them.

It is worth noting that the rates of morphological reactions are different in different parts of the uterine wall, particularly in sexually mature rats.

Specialized granulocytes move quickly out of the vessels of the serosa and the superficial layers of the uterus which line the uterine cavity, and they are actively phagocytic, as is well shown in the figure (B). In the deep layers of the uterine wall, these reactions appear considerably later, although the tissues here are in contact with the foreign body over some distance.

Our data, though preliminary, nevertheless indicate that in the uterine wall of the white rat significant changes in the character of phagocytic reactions are observed with age and with functional states of the sexual apparatus. In studying the roles of individual components of inflammation in form-producing processes in the uterine wall, we must keep in mind the significance not only of leukocytes and macrophages, but also of erythrocytic extravasations and of various complex processes observed in pregnancy and particularly postpartum.

Studies of the reactivity and the possible regenerative reconstruction of uterine wall tissues in the white rate under various physiological conditions provides supplementary material for the analysis of physiological regeneration and of pathological changes of this organ.

SUMMARY

The author studied the course of aseptic inflammation produced by passing a foreign body (sterile cotton thread soaked in carmine) through all the layers of the uterine wall in the white rat.

The phagocytic and inflammatory reactions of the uterine wall showed highly distinctive characteristics in the following groups of rats: 12 days old, $1^1/_2$ months old, sexually mature (in estrus), pregnant (7th-14th day), and immediately postpartum. The rate of formation of the connective tissue capsule was different at various stages of ontogenesis, as was the rate of appearance of giant cells.

Of great importance in the regenerative and inflammatory reactions of uterine wall tissues (especially in 12-day-old rats) are erythrocytic extravasations, which cause temporary tissue disintegration with subsequent rapid regeneration of the injured areas.

LITERATURE CITED

[1] I. G. Mikhailova, Abstracts of Reports to the Scientific Conference in Honor of the 75th Anniversary of I. I. Mechnikov's Theory of Phagocytosis (Odessa, 1958), p. 47 [In Russian].

[2] B. P. Tokin, The Immunity of Embryos (Leningrad, 1955) [In Russian].

[3] C. R. Austin, J. Endocrinol. 14, 335 (1957).

[4] W. G. Black, G. Otto, and L. E. Casida, Endocrinology 49, 237 (1951).

[5] M. Henderson, Proc. Roy. Soc. Ser. B. 142, 88 (1954).

[6] V. E. Jones, Quart. J. Micr. Sc. 88, 479 (1947).

A NEW HEAD-HOLDER FOR CATS

R. A. Durinyan and A. I. Bartyzel'

Institute of Normal and Pathological Physiology (Dir.—Active Member AMN SSSR, V. N. Chernigovskii), AMN SSSR

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

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In work with experimental animals, fast and reliable fixation is needed.

As far as fixation of the extremities is concerned, it is well known that this problem has a simple solution—the paws are tied to the operating table, and this seems to satisfy everybody. But the problem of fixation of the head is far from being solved.

The old type of head-holder customarily found in experimental laboratories has not proven satisfactory, and the vast majority of experimenters no longer use it.

This head-holder, as everyone knows, consists of a half-ring worn on the neck, and a movable ring that fixes the facial part of the head. The shaft of the head-holder is fastened to the operating table by an ordinary clamp.

It seems to us that one of the major defects of this head-holder is that it greatly complicates manipulations on the animal's head and neck. It is not an easy matter for the experimenter to ignore this. Among other defects, we might mention that when the animal moves, its head frequently comes out of the head-holder. The animal can be fixed in only one position—on its back—which decidedly reduces the instrument's usefulness.

At present the most widely used method for fixing the head is the following: An ordinary nail or something of the sort is placed in the animal's mouth, and its jaws are then tied tightly to the nail; nail and head together are then fastened to the operating table.

In a comparison with the older method, this method of fixation even possesses certain advantages: With this method of fixation, access to the neck remains free, and it is possible to fix the animal in any position, which is very important.

But even this method is not without several major objections—specifically, that with this type of fixation the animal's respiration is severely hampered, fixation sometimes requires considerable time, and cases are not infrequent in which the animal is able to free itself from this "head-holder" with abrupt movements.

These considerations motivated us to design and construct a new model of a head-holder for cats. Our principal objectives were reliability of the instrument in actual use and relative ease of construction.

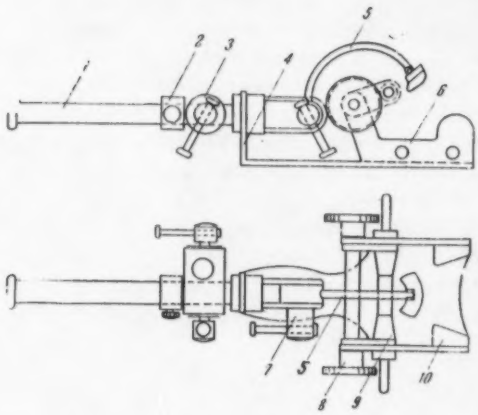

Fig. 1. Diagram of head-holder (1/2 natural size). 1) Arm; 2) connector for fixation; 3) angle connector for fastening arm to support; 4) base of head-holder; 5) nosepiece; 6) side plate; 7) fastener for nosepiece; 8) fastener for jaw clamp; 9) jaw clamp; 10) rest for lower jaw.

As may be seen from Fig. 1, this head-holder consists of an L-shaped brass plate (4) to which all other parts are fastened. The horizontal portion of this plate is shaped like a truncated cone with a semicircular indentation in its upper part. A shaped support made of plastic, where the lower jaw rests, is attached to the wide part of the plate. This support is so shaped as to restrict movements of the lower jaw in the lateral plane. At the sides of this support, brass plates (6) are held in place with screws, and the axis of rotation of a movable shaft (9) is held by these plates. The ends of this axis come out 12-15 mm from the sides, and fasteners (8) for the clamp are threaded on them; when these fasteners are tightened, the movable shaft is firmly fixed in a given position. The movable shaft serves for fixing the lower jaw. The shaft is placed behind the canine teeth, where it pins the lower jaw to the support and fixes it firmly.

For greater reliability the head-holder is also equipped with a device for fixing the upper jaw. This attachment has a holder in which a brass disk rotates, which in turn supports an arching arm with a molded nosepiece (5). On the axis of the holder there is a screw fastener (7), which fixes the disk and its nosepiece firmly when it is tightened. The nosepiece is shaped in such a way that it surrounds the nose and, resting on the zygomas, fixes the upper jaw, holding it down.

The holder for this attachment is fastened to the vertical part of the L-shaped plate by the arm of the head-holder. The arm itself is held by a connector (3) which rides on the horizontal arm of the operating table. The horizontal arm, in turn, is held by two connectors to vertical stands on the operating table (Fig. 2). By this means it is possible to raise and lower the head-holder, to move it to the left or right, or forward and backward, and finally, to rotate it 360°. The head-holder can be fastened to an ordinary operating table with a single vertical stand, with similar success. All manipulations are readily carried out on the fixated head of the animal.

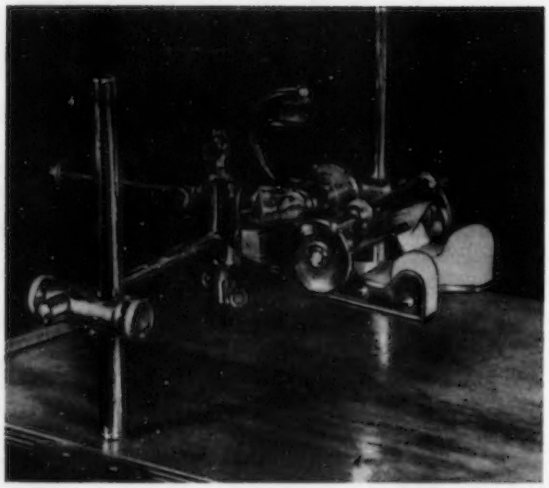

Fig. 2. Over-all view of head-holder fastened to the operating table.

As is evident from the description, the head-holder is simple to build and can be made in any workshop. With its help the head can be quickly and dependably fastened down. The head-holder absolutely does not interfere with in the neck region or on the head of the animal. It must be emphasized that the head-holder does not traumatize the tissues and can be used successfully in various operations in chronic experiments.

A SIMPLE METHOD FOR PHONOCARDIOGRAPHY IN SMALL LABORATORY ANIMALS

I. F. Mineev and V. K. Sel'tser

Laboratory of General Radiation Hygiene (Head—Cand. Med. Sci. Yu. K. Kudritskii), Institute of Radiation Hygiene (Dir.—Corresponding Member AMN SSSR N. F. Galanin)

(Presented by Active Member AMN SSSR V. V. Parin)

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Numerous publications have appeared recently dealing with studies of sound phenomena in the human heart, but the problems of phonocardiography in laboratory animals have been treated in altogether inadequate fashion in the literature.

Phonocardiography is a simple and available method for recording cardiac activity, permitting us to obtain an objective idea of the rhythm of cardiac contractions, the duration of the cardiac cycle, the principal phases of cardiac contraction (systole and diastole), and the character and intensity of heart sounds and murmurs.

The method of phonocardiography is especially valuable in experimental studies, since it allows observations to be made on the cardiac activity of any animal, without subjecting it to operative interference.

The method of phonocardiography which we use is extremely simple and available to any physiological laboratory, and permits quite accurate measurement of the duration of the cardiac cycle, systole, diastole, heart sounds—and murmurs, if there are any.

The device which picks up the sound vibrations and transforms them into electrical impulses is an electromagnetic telephone transmitter. We have used the following types: a) "TON-1 octave," with an impedance of 2,200 ohms; b) "TON-1 octave," with an impedance of 50 ohms, and c) a miniature type—"TG-7," with an impedance of 50 ohms.

The transmitter, with its diaphragm directly exposed, is applied to the body of the animal being examined at the point where it is desired to record the sound. It is not necessary to remove the hair to record the sound.

When the animal is fastened in its stand (operating table) on its abdomen, the transmitter is conveniently secured in a special opening in the table, such that the diaphragm stands $1\text{-}^1/_2$ mm above the surface of the stand. Contact of the animal's body with the transmitter diaphragm is adequate and constant during the experiment, on account of the animal's weight and fixation.

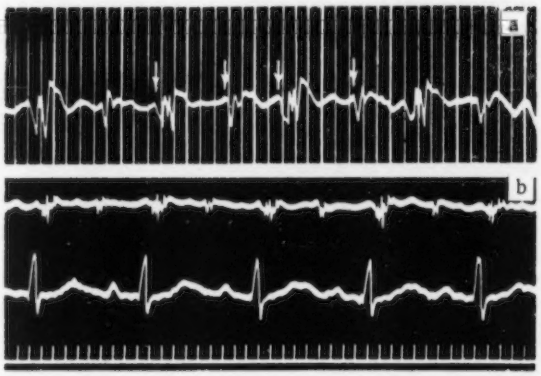

Fig. 1. a) Phonocardiogram of rabbit. Recorded with ÉKPS; pickup — telephone transmitter TG-7, 50 ohms; time marker, 20 milliseconds. b) Phonocardiogram (above) and electrocardiogram (below) of rabbit. Recorded with MPO-2; pickup — telephone transmitter TON-1, 50 ohms; time marker, 20 milliseconds.

When the animal is fixed in the supine position, the transmitter is conveniently fastened with a simple cloth belt tied with laces.

To improve and stabilize the fixation of the transmitter, one can sew a rubber cuff into the belt, to which slight additional pressure, not more than 100 mm of water, is applied after the belt is tied.

Transmitter "TON-1," with an impedance of 2,200 ohms, and the cable to the amplifier, must be shielded. Low-impedance transmitters and their cables may be left unshielded.

The electrical impulses from the transmitter are passed directly to the input of the amplifier without being filtered. We have used, with equal success, an ÉKPS electrocardiograph (Fig. 1, a) and a system consisting of a UIPP-2 DC amplifier (with alternative AC

and coupling), an MPO-2 eight-loop oscillograph (Fig. 1, b). In the latter case the signal, amplified five to ten thousand times, was supplied to the loop oscillograph through the low-impedance output.

Use of the type UIPP-2 amplifier and the MPO-2 oscillograph permits simultaneous recording of the electrocardiogram, which simplifies the interpretation of the phonocardiogram and extends the range of the investigation.

In the opinion of most investigators, the heart sounds of man are produced by vibrations of about 100 cps: According to N. N. Savitskii, the frequency is 40-80 cps [2]; according to I. I. Savchenko, 50-100 cps [3]; and according to A. I. Koblents-Mishke, not greater than 100 cps [1].

The heart sounds of laboratory animals consist of vibrations whose frequencies are on the same order.

Our pickup transmitter (TON-1 and TG-7), if fastened to the animal in a fixed position and tightly enough transform sound vibrations with frequencies of from 80 to 300 cps without perceptible distortion of amplitude relationships. The phonocardiograms of various animals are registered quite distinctly (rabbit—Fig. 1; white mouse white rat, guinea pig, and kitten—Fig. 2).

Determination of the first and second sounds on the phonocardiogram ordinarily presents no difficulties. In all the animals investigated (rabbits, guinea pigs, cats, white rats, mice), as a rule, the phonogram of the first sound is more complex, contains more waves, and has a lower frequency characteristic, greater wave amplitude, and longer duration.

With our method, sound phenomena of all frequencies picked up by the transmitter and recording system are recorded.

For most chronic experiments on animals it seems useless to us to complicate the method by including filters for separate recording of sound vibrations of different frequencies. The use of filters is necessary for investigating heart murmurs and for finer differentiation of the auditory phenomena of the heart.

Prolonged experience (a year) with the use of this method allows us to recommend it as simple and dependable for work with laboratory animals.

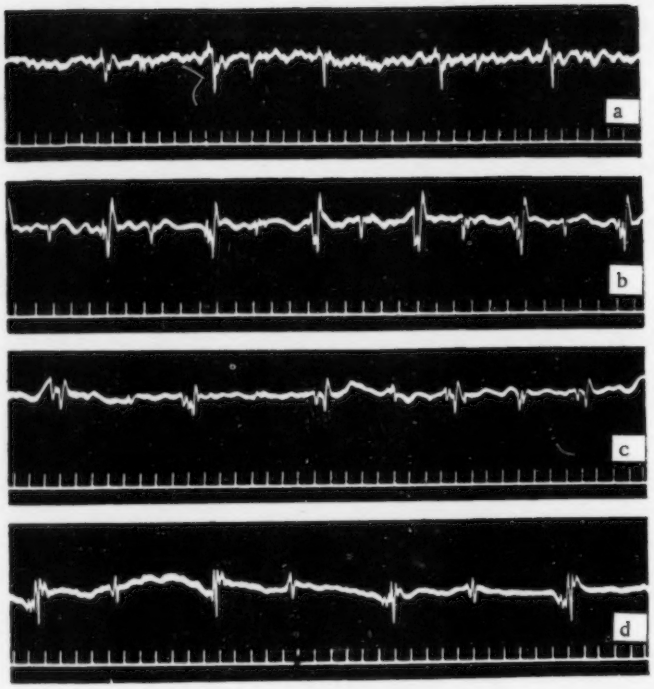

Fig. 2. Phonocardiograms of small laboratory animals. a) White mouse; b) white rat; c) guinea pig, two days old; d) kitten, two weeks old. Recorded with a MPO-2; pickup — TON-1 transmitter, 50 ohms; time marker, 20 milliseconds.

SUMMARY

The author presents a simple method of phonocardiography with various telephone transmitters (50 and 2200 ohms resistance) employed as pick-up. The phonocardiograms were recorded with the aid of an electrocardiograph or a loop oscillograph.

LITERATURE CITED

[1] A. I. Koblents-Mishke, in the book: Materials for Sharing Experience and Achievements on Scientific Frontiers 2 (27), p. 3 (Moscow, 1958) [In Russian].

[2] N. N. Savitskii, The Heart: Methods of Examination and Diagnosis (Moscow-Leningrad, 1929) [In Russian].

[3] I. I. Savchenko, Bestnik Rentgenol. i Radiol. 2, 69 (1952).

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

* BRITISH-AMERICAN TRANSLITERATION SYSTEM.

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Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Eksptl. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyateinosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

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